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FETAX is a 96-h whole embryo developmental toxicity screening assay that can be used in ecotoxicology and in detecting mammalian developmental toxicants when an *in vitro* metabolic activation system is employed. A standardized ASTM guide for the conduct of FETAX has been published. As part of the ASTM process, a three phase interlaboratory validation study was undertaken to evaluate the repeatability and reliability of FETAX. Seven different laboratories participated in the study. In Phase I, FETAX proved to be more repeatable and reliable than many other bioassays. However, some excessive variation was observed in a few laboratories. Some of this variation may have been due to an initial lack of experience with the assay by some technicians. Phase II showed far less variability than Phase I. Nonteratogens showed the most consistent results while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all FETAX endpoints ranged from 7.3 to 54.7. Phase III, which is reported here, used coded samples and test concentration ranges selected by each laboratory. Phase III yielded results similar to Phase I. Analysis of the causes of variation suggested that some technicians judged malformations more severely than others and that the ranges of concentrations tested by some of the laboratories varied greatly. A new protocol for selecting concentrations was written to reduce variation from this source. Testing to date suggests that FETAX is a repeatable and reliable bioassay.

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Abstract - The Frog Embryo Teratogenesis Assay- *Xenopus* (FETAX) is a 96-h whole embryo developmental toxicity screening assay that can be used in ecotoxicology and in detecting mammalian developmental toxicants when an *in vitro* metabolic activation system is employed. A standardized American Society for Testing and Materials (ASTM) guide for the conduct of FETAX has been published along with a companion atlas that helps in embryo staging and identifying malformations. As part of the ASTM process, a three phase interlaboratory validation study was undertaken to evaluate the repeatability and reliability of FETAX. Seven different laboratories participated in the study. In Phase I, FETAX proved to be more repeatable and reliable than many other bioassays. However, some excessive variation was observed in a few laboratories. Some of this variation may have been due to an initial lack of experience with the assay by some technicians. Phase II showed far less intra- and interlaboratory variability than Phase I. Nonteratogens showed the most consistent results while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all FETAX endpoints ranged from 7.3 to 54.7. Phase III, using coded samples and test concentration ranges selected by each laboratory showed results similar to Phase I. Analysis of the causes of variation suggested that some technicians judged malformations more severely than others and that the ranges of concentrations tested by some of the laboratories varied greatly. A new protocol for selecting concentrations for initial testing were written to reduce variation from this source. Testing to date suggests that FETAX is as repeatable and reliable as other standard bioassays.

INTRODUCTION

FETAX (Frog Embryo Teratogenesis Assay- *Xenopus*) is a 96-h whole embryo developmental toxicity test that utilizes the embryos of the South African clawed frog, *Xenopus laevis*. FETAX was initially designed as an indicator of potential human developmental health hazards and this has been enhanced by the development of an *in vitro* metabolic activation system using Aroclor 1254- and isoniazid-induced rat liver microsomes.¹⁻⁹ FETAX has undergone extensive validation using single chemicals of known mammalian developmental toxicity and additional work is currently being done.¹⁰

FETAX is also applicable to aquatic toxicity assessments and is well suited for testing complex mixtures such as industrial effluents.¹¹⁻¹⁴ This amphibian developmental toxicity test may help in studies designed to discover the reasons for the reported world-wide disappearance of amphibians even in pristine locations.^{15,16} This decline may be due in part to normal population fluctuations caused by climatological factors or by anthropogenic factors.¹⁷ However, in at least one case, frog eggs failed to develop in pond water but developed normally when moved to the laboratory.¹⁸ It is, therefore, possible that some decline may be due to chemical pollution and FETAX may be used to investigate the extent and causes of the decline.

An American Society for Testing and Materials *New Standard Guide for the conduct of FETAX* was published along with a companion *Atlas of Abnormalities* that helps in embryo staging and identifying malformations.¹⁹⁻²⁰ As part of the ASTM process, an interlaboratory validation study (ILS) was undertaken to determine the repeatability and reliability of FETAX. A three phase experimental plan with seven participants was designed. Phase I was a training and protocol evaluation phase in which the identity of the three test materials was known. Because they had been previously tested in FETAX, the same concentrations needed to establish the 96-h LC50 and EC50 (malformation) were used by all laboratories. Phase I²¹ showed that proper technician training was important in providing repeatable and reliable data. Based on Phase I results the following protocol modifications were necessary: 1) a 10% malformation rate in FETAX solution controls is acceptable, instead of 7% as specified in the ASTM Guide, 2) a change from separately pipetting toxicant and diluent directly into dishes to a procedure where toxicant and diluent are mixed in 25 ml volumes in 50 ml flasks prior to aliquoting into dishes and, 3) the elimination of 6-aminonicotinamide as the positive control. Phase I results in terms of variability and correspondence to historical data were very good with only occasional high variation observed in some laboratories. Phase II was designed to be similar to Phase I except that the identity of the test materials was not

known²². All technicians had greater experience in Phase II than in Phase I and the nature of the test compounds may have played a role in the results obtained. Phase II showed far less intra- and interlaboratory variability than Phase I. Nonteratogens showed the most consistent results while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all FETAX endpoints ranged from 7.3 to 54.7%. The most variable endpoint was the MCIG and the least variable was the LC50. Phase III, reported here, was designed to test FETAX using six test compounds not previously tested in FETAX in a blind testing format with each laboratory responsible for determining the concentrations to be tested. The *in vitro* metabolic activation system for FETAX was not employed in either Phases I, II or III.

MATERIALS AND METHODS

Interlaboratory Study Organization

Each laboratory participating in this interlaboratory study included a principal investigator and, with the exception of Dr. Doug Dawson, one or two primary technicians. At least six different laboratories tested each compound in the study. Each laboratory utilized one technician with the exception of one laboratory which utilized two independent technicians. For ease of discussion, we will refer to seven laboratories being used in total. The technicians performed FETAX while the principal investigators compiled and reported the data to a central coordinator. The experimental results were screened by the coordinator to see that they complied with the standard protocol established in the ASTM Standard Guide ¹⁹ as well as the modifications made to the standard protocol following Phase I.

Interlaboratory Study Phase III Procedure

Tests were performed as specified in the ASTM Standard Guide ¹⁹. All technicians generated their own concentration ranges based on range-finding studies as outlined in the Standard Guide¹⁹. Technicians did not know which chemical they were testing as all samples were coded. Phase I modifications were used as previously mentioned ²¹.

Samples of test solutions were retained from selected concentrations and were frozen in specially supplied borosilicate glass tubes with Teflon-lined lids (Fisher®, Dallas, TX). At the end of the experiment, the samples from each laboratory were shipped on dry ice to Dr. Bantle's laboratory for concentration analysis in the event that the cause of excess variability had to be identified. Embryos preserved in 3% v/v formalin were stored in glass scintillation vials (RPI®, Elkhart, IN) and were available to be sent to Dr. Bantle's laboratory for verification of results when necessary.

Assay Procedure

Xenopus culture, breeding procedures, and egg sorting were described previously in the ASTM Standard Guide ¹⁹ and the Atlas of Abnormalities ²⁰. Adult *Xenopus laevis* frogs were obtained from Xenopus I (Ann Arbor, Michigan).

Test Compounds

Chemicals tested in Phase III were monosodium glutamate (CAS # 6106-04-3), ascorbic acid (CAS # 50-81-7), β -aminopropionitrile (CAS # 2079-89-2), sodium acetate (CAS # 6131-90-4), sodium arsenate (CAS # 10048-95-0), and copper sulfate (CAS # 7758-99-8). The chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) in bulk quantities from the same lot. One technician individually weighed the compounds using a calibrated analytical balance. The amount was then placed in 100 ml serum vials (Fisher) and capped with rubber septa and sealed with aluminum lids. Each vial contained enough material to prepare test solutions for each 24-h interval of the experiment; four vials were needed for each test. The chemicals were sent to a coordinator who coded the samples and then shipped enough chemical to each laboratory for three definitive concentration-response experiments. Instructions shipped with each chemical included: 1) the stock concentration, 2) concentrations from which to take samples for chemical analysis, and 3) a Material Safety Data Sheet, available for use in an emergency, sealed in an envelope to prevent disclosure of the identity of the substance.

Data Analysis

Probit analysis, using the method of Litchfield-Wilcoxon ²³ was used to determine the 96-h LC50 (median lethal concentration), 96-h EC50 (concentration inducing malformations in 50% of the surviving embryos), and 95% percent confidence intervals. When the homogeneity test failed, either the trimmed Spearman-Kärber ²⁴ or EPA probit method was used instead of the Litchfield-Wilcoxon probit analysis. Teratogenic hazard was determined using a Teratogenic Index [TI=LC50/EC50 (malformation)]. Head-tail length (growth) was measured using an IBM-compatible computer equipped with digitizing software (eg. Jandel Scientific®, Corte Madera, CA). For each test, the Minimum Concentration to Inhibit Growth (MCIG) was calculated using the t-Test for grouped observations ($p < 0.05$).

The coefficient of variation (CV) values for the 96-h LC50, EC50, TI and MCIG was calculated according to Steel and Torrie ²⁵. Analysis of the data was also carried out using ASTM E691-87 (Interlaboratory Study) software and methods ²⁶.

BODY

RESULTS

Monosodium Glutamate

Three complete concentration-response tests (replicates) were performed by each laboratory. The 96-h LC50, 96-h EC50, and MCIG were determined for each replicate. All laboratories reported data except for Laboratory 3 which had difficulty in obtaining data for this compound. Monosodium glutamate often permitted the growth of microorganisms which increased variability. Intralaboratory CV values for the 96-h LC50 ranged from 2.9 to 54% and individual LC50 values ranged from a low of 0.48 to a high of 16.44 mg/ml (Table 1). The 96-h EC50 intralaboratory CV values ranged from 7.4 to 84.3%. Individual EC50 values ranged from 0.08 to 13.96 mg/ml and mean TI values ranged from 1.2 to 15.4, but the intralaboratory CV values for the mean TI were from 8.2 to 449.2%. The intralaboratory CVs for the MCIG ranged from 0 to 117.9% and the mean MCIG values ranged from 0.38 to 7.67 mg/ml.

Fig. 1 shows a graphic analysis using ASTM methodology ²⁶. Intralaboratory variability (repeatability) was given by the k values (Fig. 1A). The k value is the intralaboratory consistency statistic found by dividing the cell standard deviation for one laboratory by the repeatability standard deviation of the material ²⁶. The repeatability standard is calculated by dividing the sum of the squared cell standard deviations by the number of laboratories. The ASTM software ²⁶ generates a value for the limits of variability which is represented by a solid horizontal line(s) in each figure. Only values above the solid horizontal line on the figure were considered to have deviated excessively and only Laboratory 1 showed excessively high variation for the MCIG. The h values presented in Fig. 1B shows the interlaboratory variation (reliability). The h value is the interlaboratory consistency statistic found by dividing the cell deviation (i.e., the deviation of the cell average from the average of the cell averages) divided by the standard deviation of the cell averages ²⁶. All interlaboratory measurements were well within acceptable limits (solid horizontal lines on Fig. 1B).

Ascorbic Acid

Laboratory 6 experienced a pH problem with this compound and elected to discontinue testing since its participation was voluntary and uncompensated at this time. Intralaboratory CV values for the 96-h LC50 ranged from 4.6 to 39.5% and individual LC50 values ranged from a low of 1.06 to a high of 16.88 mg/ml (Table 2). The 96-h

EC50 CV values ranged from 2.4 to 14.1%. Individual EC50 values ranged from 0.70 to 13.68 mg/ml and mean TI values ranged from 1.0 to 2.5, while the intralaboratory CV values for the mean TI were from 1.5 to 31.5%. The intralaboratory CVs for the MCIG ranged from 5.7 to 35.4% and the mean MCIG values ranged from 0.37 to 14.67 mg/ml.

Figure 2 shows the ASTM graphic analysis method for ascorbic acid²⁶. Intralaboratory variability was given by the k values (Fig. 2A). None of the values were above the solid horizontal line and were not considered to have deviated excessively. The h values presented in Fig. 2B show the interlaboratory variation. In this case, none of the laboratories exceeded the limits of allowable variation (solid horizontal line)²⁶.

β -Aminopropionitrile

Intralaboratory CV values for the 96-h LC50 ranged from 7.0 to 112.8% and individual LC50 values ranged from a low of 2.9 to a high of 105.0 mg/L (Table 3). The 96-h EC50 CV values ranged from 5.2 to 66.2%. Individual EC50 values ranged from 0.04 to 2.22 mg/L and mean TI values ranged from 4.5 to 1221.0, while the intralaboratory CV values for the mean TI were from 12.2 to 213.6%. The intralaboratory CVs for the MCIG ranged from 14.1 to 86.9% and the mean MCIG values ranged from 0.08 to 11.73 mg/L.

Fig. 3 shows the ASTM graphic analysis method for β -Aminopropionitrile. Intralaboratory variability indicated by the k values in Fig 3A showed excess variation for the TI (Laboratory 1), the MCIG (Laboratory 2) and the EC50 (malformation) (Laboratory 4). The h values presented in Fig. 3B shows the interlaboratory variation. Laboratory 1 exceeded the ASTM variation limit²⁶ for the TI while Laboratory 2 exceeded the limit for the MCIG.

Sodium Acetate

Intralaboratory CV values for the 96-h LC50 ranged from 2.2 to 19.5% and individual LC50 values ranged from a low of 5.36 to a high of 12.32 mg/ml (Table 4). The 96-h EC50 CV values ranged from 4.8 to 33.8%. Individual EC50 values ranged from 1.08 to 9.23 mg/ml and mean TI values ranged from 0.9 to 7.5, while the intralaboratory CV values for the mean TI were from 3.3 to 44.8%. The intralaboratory CVs for the MCIG ranged from 0 to 84.1% and the mean MCIG values ranged from 0.5 to 9.33 mg/ml.

Fig. 4 shows the ASTM graphic analysis method for sodium acetate²⁶. Intralaboratory variability indicated by the k values showed that variation was acceptable

(Fig 4A). The h values presented in Fig 4B show that none of the laboratories had variation exceeding the ASTM variation limits (solid horizontal lines) ²⁶.

Sodium Arsenate

Laboratory 4 withdrew from the project after losing critical staff. Laboratory 6 provided the data for the six laboratory minimum required by the ASTM software. Intralaboratory CV values for the 96-h LC50 ranged from 6.6 to only 22.0 % and individual LC50 values ranged from a low of 1.53 to a high of 3.65 mg/ml (Table 5). The 96-h EC50 CV values ranged from 9.9 to 38.1%. Individual EC50 values ranged from 0.21 to 2.01 mg/ml and mean TI values ranged from 1.4 to 7.0, while the intralaboratory CV values for the mean TI were from 9.8 to 47.3%. The intralaboratory CVs for the MCIG ranged from 0 to 82.7% and the mean MCIG values ranged from 0.47 to 1.5 mg/ml.

Fig. 5 shows the ASTM graphic analysis method for sodium arsenate ²⁶. Intralaboratory variability indicated by the k values showed that variation was acceptable (Fig 5A). The h values presented in Fig 5B show that none of the laboratories had variation exceeding the ASTM variation limits (solid horizontal lines) although the MCIG for laboratory 7 was on the limit line.

Copper Sulfate

Intralaboratory CV values for the 96-h LC50 ranged from 5.1 to 32.8% and individual LC50 values ranged from a low of 0.29 to a high of 2.74 mg/L (Table 6). The 96-h EC50 CV values ranged from 2.7 to 74.5%. Individual EC50 values ranged from 0.08 to 3.95 mg/L and mean TI values ranged from 0.8 to 5.6, while the intralaboratory CV values for the mean TI were from 3.4 to 57.5%. The intralaboratory CVs for the MCIG ranged from 12.9 to 84.1% and the mean MCIG values ranged from 0.04 to 0.92 mg/L.

The ASTM graphic analysis method for copper sulfate is shown in Fig. 6 ²⁶. Intralaboratory variability indicated by the k values showed that variation was generally acceptable except for the EC50 (malformation) for laboratory 5 which exceeded the limit considerably (Fig 6A). The h values presented in Fig 6B show that none of the laboratories had variation exceeding the ASTM variation limits (solid horizontal lines) ²⁶.

Interlaboratory Variability as Determined by CV Values

Table 7. shows interlaboratory summary results for all six test compounds. CV values above 75% were considered somewhat high while values above 150% were considered excessive based on bioassay results reported by Parkhurst et al. 27. Interlaboratory CV values were very good except for monosodium glutamate (84.5% and β -aminopropionitrile (108.2%). For the EC50 (malformation), three of the six test compounds produced somewhat high results (monosodium glutamate- 134.9%, β -aminopropionitrile-85.1% and copper sulfate- 111.4%). Except for ascorbic acid, most of the values for the TI were either high or excessive. CV values for the MCIG were better with values for ascorbic acid, sodium acetate and sodium arsenate better within reasonable limits (Table 7).

CONCLUSIONS

Monosodium Glutamate

Monosodium glutamate has not been previously tested in FETAX. Previous reports suggest little developmental toxicity attributable to monosodium glutamate administered as a brief treatment in mice²⁸. Monosodium glutamate was typical of compounds with low developmental toxicity tested in the FETAX ILS in that overall variation was fairly low for this study. This was similar to Phase II findings²². The chief cause of the observed interlaboratory variation was Laboratory 5 which reported very low LC50 (Laboratory mean 0.56 mg/ml) and EC50 (malformation) (Laboratory mean 0.24 mg/ml) values while Laboratory 6 reported LC50 and EC50 (malformation) values of 15.96 and 12.96 mg/ml, respectively. The interlaboratory means were 6.52 and 3.43, respectively. A greater problem was that the mean laboratory TI ranged from a low of 1.19 which indicated little teratogenic hazard to a high of 15.4 which suggested strong teratogenic hazard. The intralaboratory variation was quite low suggesting other factors were affecting interlaboratory variability. There were some reports of bacterial contamination when this compound was tested. Some test compounds permit or even support the growth of microbial organisms which can then affect test results. Antibiotic treatments were avoided in this study but may be used as long as suitable controls are used to account for possible interactions¹⁹. Laboratory 3 was unable to successfully test this compound, perhaps because of contamination.

Ascorbic Acid

Previous FETAX tests with ascorbic acid indicated LC50, EC50 (malformation) and MCIG values of 19.7, 12.13 and 10 mg/ml. The TI was 1.62. Table 7 shows that the six laboratories that tested ascorbic acid in the ILS study had interlaboratory mean values of 10.70, 7.61 and 6.28 mg/ml, respectively, for the LC50, EC50 (malformation) and MCIG. The TI was 1.4. The lot numbers of ascorbic acid were different between the trials and the ILS numbers were reduced by Laboratory 4 who reported very low values for the LC50, EC50 (malformation) and MCIG endpoints (Table 2). The reason for these low values is unknown. Interestingly, the resultant TI was not greatly different from the other laboratories. On the whole, results were judged to be very good. Ascorbic acid was considered to pose little developmental hazard and this was confirmed in this study. The relationship of these tests results to the mammalian literature has already been discussed²⁹.

β -Aminopropionitrile

This chemical has not been previously tested in FETAX although the mammalian literature indicates that it is a lathrygen in rats (^{30,31}). Results from this study seem to confirm this as a TI value of 55.3 was reported (Table 7). The LC50, EC50 (malformation) and MCIG values were 34.76, 0.63 and 2.11 mg/L, respectively. Once again, there was a fairly significant range observed between laboratories, especially for

the LC50 (Table 3). For example, Laboratories 1 and 7 reported values near 80 mg/L, while Laboratories 3, 5 and 6 reported values at least 10 fold lower. Interestingly, the EC50 (malformation) endpoint was less variable and these differences caused great variability in the TI as seen in the high interlaboratory CV value of 991.6% (Table 7).

Sodium Acetate

Sodium acetate has been previously tested in FETAX but the exposure period was for 5 days instead of the standard four and, therefore, the data was not directly comparable to this study. The five day exposure data was 4.2, 3.3 and 2.5 mg/ml for the LC50, EC50 (malformation) and MCIG, respectively (³²). The TI was 1.3. Predictably, the results of the four day exposure study was higher at 9.26, 5.00 and 4.48 mg/ml for the LC50, EC50 (malformation) and MCIG, respectively (Table 7). The TI was 1.9 which indicated a modest degree of teratogenic hazard. The interlaboratory variability for the LC50 and EC50 (malformation) was within acceptable ranges but the CV for the TI was high (Table 7). The CV values for TIs tended to be higher because the LC50 is divided by the EC50 (malformation) thereby causing a multiplication effect on the resultant CV values for the TI.

Sodium Arsenate

Sodium arsenate has not been previously tested in FETAX. This study found that the LC50, EC50 (malformation) and MCIG were 2.30, 0.97 and 1.11 mg/ml, respectively (Table 7). The TI was 2.4 and indicated modest teratogenic activity. Malformations were moderate to severe in nature. Interlaboratory variability was generally low with this compound except for the TI again (Table 7). Between laboratories the EC50 (malformation) proved considerably more variable than the LC50.

Mammalian studies indicated some teratogenicity in rodents such as mice ³³, hamsters ³⁴ and rats ³⁵. The FETAX studies reported here indicate less developmental hazard due to arsenate than the mammalian studies. Limited human studies of accidental exposures did not detect human developmental toxicity.

Copper Sulfate

Fort et al., ³⁶ tested copper sulfate in FETAX. They found that the mean LC50, EC50 (malformation) and MCIG were 0.94, 0.81 and 0.75 mg/L, respectively. The TI was only 1.15 which indicated little developmental hazard. Data from this study showed excellent correspondence to the historical data as the values were 1.23, 0.83 and 0.35 mg/L and the TI was 1.5 (Table 7). Most of the laboratories reported consistent results but the mean laboratory TI values ranged from 0.8 to 5.58.

The effects of copper on mammalian development has been reported by Everson et al. ³⁷ and Kalter ³⁸. Some effects on neural tissue are reported but copper is not

considered a strong teratogen. The main effects are embryotoxicity and FETAX confirms this.

Endpoint Variability

Table 8 shows that the LC50 was the least variable endpoint based on comparison of CV values in five of six tests. The MCIG was the most variable in three of six tests. These results were not surprising because the concentration ranges were designed to define the LC50 and EC50 values and the LC50 is the more objective endpoint compared to the EC50.

Sources of Variation

In analyzing the results of this study and Phases I and II, it became apparent that at least one technician typically judged malformations more harshly than any other throughout the four years of testing. This pattern was not repeated for the LC50 estimation. This occurred even though there was extensive technician training and practical experience. Another technician, reported high EC50 (malformation) and LC50 values in two of the three phases and was second highest in the third Phase. This may have been due to problems in sample dilution because the LC50 tracked the EC50 in this Laboratory.

The chief difference between Phase II which yielded outstanding results and Phase III which provide good but far more variable results was the ability of participants to choose their own test concentrations. Analysis of the concentrations selected by participants revealed that some selected very narrow ranges of close steps between concentrations while other selected far broader ranges. Different toxicants yielded concentration-response curves with different slopes and this seemed to have an effect on results depending on the concentrations selected. In an effort to standardize the selection of test concentration yet still allow each laboratory to select their own concentration the following protocol was written. While complicating the procedure, it seems to be yielding better results at this stage of the study. Further tests are now being conducted to determine whether variability is reduced.

Recommended Protocol for Testing New Compounds Using FETAX

The following sequence is now being followed when testing a new chemical for a three way round robin test program currently in progress. This procedure will help to standardize initial-range finding experiments and help reduce some of the variability found in the present study. The metabolic activation system (MAS) is composed of Aroclor 1254-induced rat liver microsomes and NADPH generator system⁵.

Initial Range-Finding Test (No MAS used)

The range-finding tests consists of a series of at least seven concentrations that differ by a factor of ten. This is adequate to delineate the concentration range to establish the 96-h LC50 and EC50 (malformation)¹⁹. Growth inhibition data are not collected from range-finding tests. Investigators are encouraged to add concentrations whenever possible to find the best approximation of the LC50 and EC50. Once the data are collected from the range-finding tests, the LC50 and EC50 should be estimated using probit analysis, trimmed Spearman-Kärber or the two point method. The two point method is used when regular statistical programs fail to generate any useful data. It involves drawing a line from the low response to the high response and the 50% response concentration is estimated from where the line intercepts the 50% response point. If the data allows the Litchfield-Wilcoxon test or trimmed Spearman-Kärber methods to be used, then Litchfield-Wilcoxon is used when the data passes homogeneity (Shapiro-Wilks) and normality (Chi Square) tests and trimmed Spearman-Kärber is used when the data fails these tests. Range-finding tests may bypass the homogeneity requirement here but not in replicate tests discussed below. The Toxstat version 3.0 program from the Univ. of Wyoming is used to make homogeneity and normality assessments (Fish Physiology and Toxicology Laboratory, Dept. of Zoology and Physiology, P.O. Box 3166, University of Wyoming, Laramie, WY, 82071).

Estimation of the unactivated LC50 and EC50 (No MAS used).

This is a one or two step process depending on the nature of the test compound and the results of the first test in this series. The test is performed using the sliding scale of concentrations presented in Table 9. The table presents values from 0.001 to 100. From 0.001 to 0.1 the steps are 0.005. From 0.1 to 1, the steps are 0.05 and from 1 to 10, the steps (differences between concentrations) are 0.5. Lastly, the steps are 5.0 from concentration 10 to 100. The value on the table closest to the LC50 is found. Then three values are chosen immediately below and three values immediately above this point. The same is performed for the EC50. A test is performed and the data collected. The LC50 and EC50 values with confidence limits are then generated. After the successful conclusion of this test, a determination is made as to whether a metabolic activation system should be used in subsequent testing. From the data obtained in above, calculate the LC5, LC16, LC50, LC84 and LC95 and the EC5, EC16, EC50, EC84 and EC95. By determining the above values, the concentrations to be tested in the definitive tests below are established and the slope of the concentration-response curves are accounted for. Additional concentrations may be added to ensure obtaining an LC50 and EC50, but the same concentrations must be used for each replicate (definitive) test.

Replicate (Definitive) Tests (MAS used).

Three tests are then performed with and without MAS each with a separate clutch of embryos. The ten concentrations determined above are used, with and without MAS. The unactivated experiments should yield good LC50 and EC50 values. If they do not,

the tests must be repeated. If the MAS test yields LC50 and EC50 values on the first test by an acceptable probit analysis method, the results should be reported. If the addition of MAS alters toxicity such that LC50 and EC50 values can not be determined, then more range finding is done similar to that for the unactivated tests above until the proper concentration range for MAS is defined. Tests with and without MAS are then performed on the same clutch of embryos until three replicate tests are obtained.

Controls to be performed for the initial range finding step and the estimation of the unactivated LC50 and EC50 (No MAS used).

Note: dishes are plastic unless otherwise noted.

- 1.) FETAX solution. Use four dishes of 20 embryos each.
- 2.) FETAX + cosolvent (if required). Use four dishes of 20 embryos each.

Controls to be performed with each replicate (definitive) test:

- 1.) FETAX/Antibiotics solution. Use four dishes of 20 embryos each.
FETAX/Antibiotics solution is the diluent for all test concentrations.
- 2.) FETAX/Antibiotics solution + cosolvents if one is used. Use four dishes of 20 embryos each. Refer to ASTM guide¹⁹ on when to use for data comparison.
If a cosolvent is required, then the following controls (except the cyclophosphamide controls) must contain the cosolvent.
- 3.) Four mg/ml cyclophosphamide positive controls with MAS. Two dishes of 20 embryos each.
- 4.) Microsomes alone (no generator) + four mg/ml cyclophosphamide. Two dishes of 20 embryos each.
- 5.) Microsomes alone (no generator) + test compound at the LC50 concentration.
2 dishes of 20 embryos each.
- 6.) Generator system only plus test compound at the LC50 concentration. Two dishes of 20 embryos each.
- 7.) MAS alone. Two dishes of 20 embryos each.

Final Conclusions

The results of this study indicated that FETAX is still a repeatable and reliable assay but that variation due to technician bias in terms of judging malformations and in selecting test concentrations must be controlled. Bacterial contamination still causes variability but this can be detected but noting sample turbidity and variation in replicates of the same concentration. Antibiotics can be used to control contamination but care must be exercised to account for possible interaction. Variability is generally lower for nonteratogens than strong teratogens.

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REFERENCES

1. J.N. Dumont, T.W. Schultz, M.V. Buchanan and G.L. Kao, Frog embryo teratogenesis assay: *Xenopus* (FETAX) - A short-term assay applicable to complex environmental mixtures. In *Symposium on the Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III*, ed. by M. D. Waters, S.S. Sandhu, J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow, pp. 393-405. Plenum Press, New York (1983).
2. J. A. Bantle and D. A. Dawson, Uninduced rat liver microsomes as an *in vitro* metabolic activation system for the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). In *Aquatic Toxicology and Hazard Assessment*, Vol. 10, ed. by W.J. Adams, G. A. Chapman and W. G. Landis, ASTM STP 971, pp. 316-326. American Society for Testing and Materials, Philadelphia (1988).
3. J. A. Bantle, D. J. Fort and B. L. James, Identification of developmental toxicants using the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). *Hydrobiol* **188/189**, 577-585 (1989).

4. D. A. Dawson, D. J. Fort, G. L. Smith, D. L. Newell and J. A. Bantle,
Evaluation of the developmental toxicity of nicotine and cotinine with Frog
Embryo Teratogenesis Assay: *Xenopus* (FETAX). *Teratogen. Carcinogen.*
Mutagen. **8**, 329-338 (1988).
5. D. J. Fort, D. A. Dawson and J. A. Bantle, Development of a metabolic
activation system for the Frog Embryo Teratogenesis Assay: *Xenopus*
(FETAX). *Teratogen. Carcinogen. Mutagen.* **8**, 251-263 (1988).
6. D. J. Fort and J. A. Bantle, Use of Frog Embryo Teratogenesis Assay -
Xenopus and an exogenous metabolic activation system to evaluate the
developmental toxicity of diphenylhydantoin. *Fund. Appl. Toxicol.* **14**, 720-
733 (1990a).
7. D. J. Fort and J. A. Bantle, Analysis of the mechanism of isoniazid-induced
developmental toxicity with the Frog Embryo Teratogenesis Assay - *Xenopus*
(FETAX), *Teratogen. Carcinog. Mutagen.* **10**, 463-476 (1990b).

8. D. J. Fort, J. R. Rayburn, D. J. DeYoung and J. A. Bantle, Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. *Drug Chem. Toxicol.* **14**(1&2), 143-160 (1991).
9. D. J. Fort, J. R. Rayburn and J. A. Bantle, Evaluation of acetaminophen-induced developmental toxicity using FETAX, *Drug Chem. Toxicol.* **15**, 329-350 (1992).
10. J. A. Bantle, FETAX - A developmental toxicity assay using frog embryos. In: *Fundamentals of Aquatic Toxicology*, 2nd ed., ed. by G. M. Rand and S. R. Petrocelli, Hemisphere Publishing, New York (1993), In Press.
11. J. N. Dumont and T. W. Schultz, Effects of coal-gasification sour water on *Xenopus laevis* embryos. *J. Environ. Sci. Health A15*, 127-138 (1980).
12. D. A. Dawson, C. A. McCormick and J. A. Bantle, Detection of teratogenic substances in acidic mine water samples using the Frog Embryo Teratogenesis Assay-*Xenopus*(FETAX). *J. Appl. Toxicol.* **5**(4), 234-244 (1985).
13. D. A. Dawson, E. Stebler, S. A. Burks and J. A. Bantle, Teratogenicity of metal contaminated sediment extracts to frog (*Xenopus laevis*) and fathead

- minnow (*Pimephales promelas*) embryos. *Environ. Toxicol. Chem.* **7**(1), 27-34 (1988).
14. J. A. Bantle, D. J. Fort and D. A. Dawson, Bridging the gap from short-term teratogenesis assays to human health hazard assessment by understanding common modes of teratogenic action. In *Aquatic Toxicology and Hazard Assessment*, Vol. 12, ed. by W. G. Landis, W. H. Van der Schalie, ASTM STP 1096, pp. 46-58. American Society for Testing and Materials, Philadelphia, (1989).
 15. D. B. Wake and H. J. Morowitz, Declining amphibian populations-a global phenomenon, report of a workshop sponsored by the Board on Biology, National Research Council, held at Irvine, CA, February 19-20, (1990).
 16. D. B. Wake, Declining amphibian populations. *Science* **253**, 860 (1991).
 17. J. H. K. Pechman, D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt and J. W. Gibbons, Declining amphibian populations: The problem of separating human impacts from natural fluctuations. *Science* **253**, 892-895 (1991).

18. Science Briefings, New task force on declining amphibians. *Science* **253**, 509 (1991).
19. American Society for Testing and Materials, *New standard guide for conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX)*, ASTM E1439-91, Special Publications Philadelphia, (1991).
20. J. A. Bantle, J. N. Dumont, R. Finch and G. Linder, *Atlas of Abnormalities: A Guide for the Performance of FETAX*. Oklahoma State Publications Department (1991).
21. J.A. Bantle, D.T. Burton, D.A. Dawson, J.N. Dumont, R.A. Finch, D.J. Fort, G. Linder, R.A. Rayburn, D. Buchwalter, M.A. Maurice, and S.D. Turley. Initial interlaboratory validation study of FETAX: Phase I testing. *Journal of Applied Toxicology*, 14(3),213-223 (1994).
22. J.A. Bantle, D.T. Burton, D.A. Dawson, J.N. Dumont, R.A. Finch, D.J. Fort, G. Linder, R.A. Rayburn, D. Buchwalter, A.M. Gaudet-Hull, M.A. Maurice and S.D. Turley. Initial interlaboratory validation study of FETAX: Phase II testing. *Environmental Toxicology and Chemistry*, 13(10), 1629-1637 (1994).

23. R. J. Tallarida and R. B. Murray, *Manual of Pharmacologic Calculations with Computer Programs*. 2nd Edn, Springer-Verlag, New York (1987).
24. M. A. Hamilton, R. C. Russo and R. V. Thurston, Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Tech.* **11**(7), 714-718 (1977).
25. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd Edn, ed. by C. Napier and J.W. Maisel. McGraw-Hall Book Company, New York (1980).
26. American Society for Testing and Materials, *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*, ASTM E691-87, Annual book of ASTM standards, **14.02**, 419-438, Philadelphia (1988).

27. B. R. Parkhurst, W. Warren-Hicks and L. E. Noel, Performance characteristics of effluent toxicity tests: Summarization and evaluation of data. *Envir. Toxicol. Chem.* **2**, 771-791 (1992).
28. U. Murakami and M. Inouye, Brain lesions in the mouse fetus caused by maternal administration of monosodium glutamate. *Congenital Anomalies* 11:171-177, 1971.
29. D.J. DeYoung, J.A. Bantle and D.J. Fort, Developmental toxicity of ascorbic acid, sodium selenate, coumarin, serotonin, and 13-cis retinoic acid tested in FETAX. *Drug and Chemical Toxicology*, 14,127-141 (1991).
30. F.W. Stamler, Reproduction in rats fed lathrus peas or aminonitriles. *Proc. Soc. Exp. Biol. Med.* 90: 294-298 (1955).

31. A.J. Steffak, A.C. Verrusio and C.A. Watkins, Cleft palate in rodents after maternal treatment with various lathyrogenic agents. *Teratology* 5: 33-40 (1972).
32. D.J. Deyoung, M.A. Hull and J.A. Bantle, Differences in sensitivity to developmental toxicants as seen in *Xenopus* and *Pimephales* embryos. *Bulletin of Environmental Contamination and Toxicology*, Submitted (1995).
33. M.N. Baxley, R.D. Hood, G.C. Vedel, W.P. Harrison, and G.M. Szezech, Prenatal toxicity of orally administered sodium arsenate in Mice. *Bulletin of Environmental Contamination and Toxicology*, 26:749-756, (1981).
34. A.R. Beaudoin, Teratogenicity of sodium arsenate in rats. *Teratology* 10: 153-158, (1974).
35. V.H. Ferm, A. Saxon and B.M. Smith, The teratogenic profile of sodium arsenate in the golden hamster. *Arch. Environ. Health* 22: 557-560, (1971).

36. D.J. Fort, E.L. Stover and J.A. Bantle, Integrated ecological hazard assessment of waste site soil extracts using FETAX and short-term fathead minnow teratogenesis assay, *Environmental Toxicology and Risk Assessment: Transboundary Issues in Pollution- Air, Surface, and Groundwater*: Fourth Volume, ASTM STP 1262, T. W. LaPoint, E.E. Little, F.T. Price, Eds., American society for Testing and Materials, Philadelphia, (1995)- In Press.
37. R.L. Brinster and P.C. Cross, Effect of copper on the preimplantation mouse embryo. *Nature* 238, 398-399, (1972).
38. F.J. DiCarlo, Syndromes of of cardiovascular malformations induced by copper citrate in hamsters. *Teratology*. 21, 89-101, (1980).

Table 1. Results of the Interlaboratory Study for Monosodium Glutamate.

Laboratory	Replicate (n)	LC50 (mg/ml)	Mean		LC50 CV1 (%)	Mean		EC50 CV (%)	Mean EC50 (mg/ml)	EC50 CV (%)	Mean TI ²	TI CV (%)	MCIG ³ (mg/ml)	Mean MCIG (mg/ml)	MCIG CV (%)
			LC50 (mg/ml)	CV1 (%)		EC50 (mg/ml)	CV (%)								
1	1	4.07				0.46							10.0		
	2	5.64	7.80	54.0	0.37	0.51	26.6	15.4	29.3				1.3	3.92	110.1
	3	13.68			0.69								0.5		
2	1	8.02				1.32							4.0		
	2	4.15	7.56	34.6	0.86	1.02	20.5	7.4	41.1				0.3	1.50	117.9
	3	10.50			0.89								0.3		
3	1														
	2														
	3														
4	1	1.35				0.08							--		
	2	1.07	1.07	21.4	1.39	0.65	84.3	1.7	449.2				0.3	0.38	33.3
	3	0.79			0.48								0.5		
5	1	0.66				0.26							0.4		
	2	0.48	0.56	13.4	0.21	0.24	9.7	2.3	8.2				0.5	0.42	5.7
	3	0.54			0.26								0.4		
6	1	16.10				11.66							7.5		
	2	16.44	15.96	2.9	13.96	12.96	7.4	1.2	8.2				7.5	7.50	0.00
	3	15.34			13.27								7.5		
7	1	5.47				3.90							10.0		
	2	6.52	6.17	8.0	5.67	5.19	17.7	1.2	11.3				7.0	7.67	22.2
	3	6.51			5.99								6.0		

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

Table 2. Results of the Interlaboratory Study for Ascorbic Acid.

Laboratory	Replicate (n)	Mean LC50		Mean EC50		Mean TI		Mean MCIG ³		Mean MCIG	
		LC50 (mg/ml)	CV1 (%)	EC50 (mg/ml)	CV (%)	TI ² (%)	CV (%)	MCIG ³ (mg/ml)	MCIG (mg/ml)	CV (%)	MCIG (%)
1	1	11.04		7.01				5.0			
	2	6.98	29.5	5.31	14.1	1.7	16.8	10.0	8.33		28.3
	3	14.91		7.48				10.0			
2	1	15.21		7.00				2.0			
	2	8.85	22.8	6.69	12.7	2.1	31.5	2.0	2.67		35.4
	3	15.11		5.17				4.0			
3	1	15.61		11.33				2.0			
	2	16.88	13.9	11.99	2.4	1.3	12.9	4.0	3.33		28.3
	3	12.00		11.49				4.0			
4	1	2.78		0.82				0.3			
	2	1.61	39.5	0.70	7.6	2.5	31.3	0.3	0.37		25.7
	3	1.06		0.70				0.5			
5	1	9.26		7.24				9.0			
	2	10.21	4.6	7.69	2.6	1.3	3.6	8.0	8.33		5.7
	3	10.25		7.35				8.0			
6	1										
	2										
	3										
7	1	12.20		11.94				15.0			
	2	14.26	7.3	13.47	6.0	1.0	1.5	16.0	14.67		8.5
	3	14.36		13.68				13.0			

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

Table 3. Results of the Interlaboratory Study for β -aminopropionitrile.

Laboratory	Replicate (n)	Mean		LC50		EC50		Mean		EC50		Mean		TI		MCIG ³		Mean		MCIG	
		LC50 (mg/L)	LC50 (mg/L)	CV ¹ (%)	CV ¹ (%)	EC50 (mg/L)	EC50 (mg/L)	EC50 (mg/L)	EC50 (mg/L)	CV (%)	CV (%)	TI ² (%)	TI ² (%)	CV (%)	CV (%)	MCIG ³ (mg/L)	MCIG ³ (mg/L)	MCIG (mg/L)	MCIG (mg/L)	CV (%)	CV (%)
1	1	93.70				0.07				25.5		1221.0		58.7		0.08		0.08		14.1	
	2	45.40	79.37	30.4		0.08		0.07								0.08		0.08			
	3	99.00				0.04										0.10					
2	1	9.50				0.021		0.30		57.9		97.9		30.9		0.20		11.73		86.9	
	2	65.40	29.43	86.6		0.55										25.00					
	3	13.40				0.15										10.00					
3	1	5.30				0.28		0.93		49.2		4.5		167.2		0.25		0.33		35.4	
	2	2.90	4.17	23.6		1.28										0.25					
	3	4.30				1.24										0.50					
4	1	12.60				2.22		1.15		66.2		35.4		213.6		0.50		0.45		59.5	
	2	105.00	40.53	112.8		0.63										0.75					
	3	4.00				0.59										0.10					
5	1	5.50				0.67										1.00		0.00133		35.4	
	2	4.70	4.90	8.8		0.66		0.69		5.2		7.1		12.2		2.00					
	3	4.50				0.74										1.00					
6	1	7.70				0.21										0.10		0.20		54.0	
	2	7.40	7.93	7.0		0.22		0.20		11.8		40.1		20.6		0.15					
	3	8.70				0.17										0.35					
7	1	60.00				1.10		1.07		11.7		72.2		30.8		1.00		0.67		35.4	
	2	76.00	77.00	18.6		1.20										0.50					
	3	95.00				0.90										0.50					

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

Table 4. Results of the Interlaboratory Study for Sodium Acetate.

Laboratory	Replicate (n)	Mean		LC50		EC50		Mean		EC50		Mean		TI		MCIG ³		Mean		MCIG	
		LC50 (mg/ml)	CV1 (%)	LC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	TI (%)	CV (%)	MCIG ³ (mg/ml)	CV (%)	MCIG ³ (mg/ml)	CV (%)	MCIG (mg/ml)	CV (%)
1	1	6.96		2.32		2.37		3.00		1.0		3.00									
	2	6.30	10.6	1.41	33.8	2.37	33.8	2.6	44.8	6.0	72.0										
	3	5.36		3.37						2.0											
2	1	11.20		2.01		1.45		7.5		0.5		0.50									
	2	11.07	2.3	1.18	27.1	1.45	27.1	7.5	22.9	0.5	0.0										
	3	10.62		1.17						0.5											
3	1	12.00		5.12		7.21		1.6		10.0		9.33									
	2	11.00	4.2	7.64	21.7	7.21	21.7	1.6	27.6	9.0	5.1										
	3	12.06		8.88						9.0											
4	1	11.69		5.23		6.12		1.6		1.0		1.67									
	2	9.92	17.4	6.62	10.3	6.12	10.3	1.6	28.3	1.0	56.6										
	3	7.56		6.52						3.0											
5	1	7.76		7.92		7.50		0.9		8.0		7.67									
	2	7.67	13.3	8.26	11.3	7.50	11.3	0.9	3.3	8.0	6.1										
	3	5.72		6.31						7.0											
6	1	12.32		8.77		8.74		1.4		5.0		5.67									
	2	11.68	2.2	9.23	4.8	8.74	4.8	1.4	5.9	5.0	16.6										
	3	11.96		8.21						7.0											
7	1	8.91		1.55		1.64		4.4		0.50		3.50									
	2	7.31	19.5	2.30	30.6	1.64	30.6	4.4	24.7	7.50	84.1										
	3	5.46		1.08						2.50											

1 CV=Coefficient of Variation.
2 TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.
3 MCIG=Minimum Concentration to Inhibit Growth.

Table 5. Results of the Interlaboratory Study for Sodium Arsenate.

Laboratory	Replicate (n)	LC50 (mg/ml)	Mean		LC50 CV1 (%)	Mean		EC50 CV (%)	Mean TI ²	TI CV (%)	MCIG ³ (mg/ml)	Mean		MCIG CV (%)
			LC50 (mg/ml)	(mg/ml)		EC50 (mg/ml)	(mg/ml)					MCIG (mg/ml)	(mg/ml)	
1	1	2.53				0.58		38.1	5.3	39.3	0.30		0.47	82.7
	2	1.87	2.09	14.9	0.21	0.40					0.10			
	3	1.87			0.40						1.00			
2	1	2.65				0.59		32.4	7.0	38.7	1.00		1.00	0.0
	2	3.65	3.03	14.6	0.45	0.43					1.00			
	3	2.79			0.25						1.00			
3	1	1.53				1.27			1.5	47.3	1.50		1.17	20.2
	2	2.03	2.07	22.0	1.91	1.40		27.1			1.00			
	3	2.64			1.01						1.00			
4	1													
	2													
	3													
5	1	1.77				1.23					1.50		1.50	0.0
	2	2.47	2.07	14.1	1.54	1.28		14.9	1.6	10.0	1.50			
	3	1.98			1.08						1.50			
6	1	2.89				2.01					1.00		1.00	0.0
	2	2.50	2.45	15.4	1.64	1.76		9.9	1.4	9.8	1.00			
	3	1.97			1.64						1.00			
7	1	1.89				0.39			4.0	13.9	1.00		1.50	47.1
	2	2.16	2.08	6.6	0.56	0.52		18.6			2.50			
	3	2.20			0.62						1.00			

1CV=Coefficient of Variation.

LCV=Coefficient of Variation.
 2π -Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIC=Minimum Concentration to Inhibit Growth.

Table 6. Results of the Interlaboratory Study for Copper Sulfate.

Laboratory	Replicate (n)	Mean LC50		Mean EC50		Mean TI ²	TI CV (%)	Mean MCIG ³		Mean MCIG (mg/L)	MCIG CV (%)
		LC50 (mg/L)	CV ¹ (%)	LC50 (mg/L)	CV (%)			MCIG ³ (mg/L)	CV (%)		
1	1	1.00		0.32				0.70			
	2	1.45	32.8	0.21	17.7	5.6	33.4	0.40		0.57	22.0
	3	2.24		0.31				0.60			
2	1	0.63		0.15				0.20			
	2	0.79	13.9	0.24	19.0	3.8	10.5	0.40		0.27	35.4
	3	0.89		0.22				0.20			
3	1	0.93		--				0.05			
	2	0.66	15.6	0.33	25.8	1.9	8.0	0.05		0.04	51.4
	3	0.95		0.56				0.01			
4	1	0.29		0.08				0.01			
	2	0.50	26.8	0.11	74.5	2.3	57.5	0.03		0.04	84.1
	3	0.58		0.41				0.08			
5	1	1.70		2.47				0.75			
	2	2.07	19.8	1.61	36.1	0.8	34.6	1.00		0.92	12.9
	3	2.74		3.95				1.00			
6	1	0.88		0.81				0.25			
	2	0.82	5.1	0.76	2.7	1.1	3.4	0.25		0.20	35.4
	3	0.93		0.80				0.10			
7	1	1.49		1.48				0.50			
	2	2.09	16.2	0.89	24.7	1.8	36.6	0.30		0.43	21.8
	3	2.20		0.92				0.50			

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

Table 7. Results of the Interlaboratory Study.

Chemical	Mean LC50 ¹		Mean EC50 ¹		Mean TI ³		Mean MCIG	
	CV ²	(%)	CV	(%)	CV	(%)	CV	(%)
Monosodium Glutamate ⁵	6.52	84.5	3.43	134.9	1.9	318.3	3.75	98.8
Ascorbic Acid ⁵	10.70	44.5	7.61	53.0	1.4	46.3	6.28	78.1
β -Aminopropionitrile	34.76	108.2	0.63	85.1	55.3	991.6	2.11	261.1
Sodium Acetate	9.26	26.2	5.00	59.4	1.9	135.8	4.48	74.4
Sodium Arsenate	2.30	21.7	0.97	59.7	2.4	120.8	1.11	44.5
Copper Sulfate	1.23	54.6	0.83	111.4	1.5	127.3	0.35	87.2

¹All values are mg/ml except β -aminopropionitrile and copper sulfate which are mg/L.

²CV=Coefficient of Variation.

³TI=Mean Teratogenic Index calculated by dividing the mean LC50 by the mean EC50 for all laboratories.

⁴MCIG=Minimum Concentration to Inhibit Growth.

⁵ Only six laboratories reported.

Table 8. Comparison of the Phase III Interlaboratory Variability of FETAX Endpoints Based on CV Values.

Compound	Least Variable	Mid-Range	Most Variable
Monosodium Glutamate	LC50	MCIG	EC50
Ascorbic Acid	LC50	EC50	MCIG
β -Aminopropionitrile	EC50	LC50	MCIG
Sodium Acetate	LC50	EC50	MCIG
Sodium Arsenate	LC50	MCIG	EC50
Copper Sulfate	LC50	MCIG	EC50

Table 9. Concentration Series to be Used in the First Round of Testing.

Low Series	Medium Series	High Series
0.001	0.10	20
0.005	0.15	25
0.010	0.20	30
0.015	0.25	40
0.020	0.30	45
0.025	0.35	50
0.030	0.40	55
0.035	0.45	60
0.040	0.50	65
0.045	0.55	70
0.050	0.60	75
0.055	0.65	80
0.060	0.70	85
0.065	0.75	90
0.070	0.80	95
0.075	0.85	100
0.080	0.90	
0.085	0.95	
0.090	1.0	
0.095	1.5	
0.10	2.0	
0.15	2.5	
0.20	3.0	
0.25	3.5	
0.30	4.0	
0.35	4.5	
0.40	5.0	
0.45	5.5	
0.50	6.0	
0.55	6.5	
0.60	7.0	
0.65	7.5	
0.70	8.0	
0.75	8.5	
0.80	9.0	
0.85	9.5	
0.90	10	
0.95	15	

FIGURE LEGENDS

Figure 1. Intralaboratory k and interlaboratory h values for monosodium glutamate. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

Figure 2. Intralaboratory k and interlaboratory h values for ascorbic acid. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

Figure 3. Intralaboratory k and interlaboratory h values for β -aminopropionitrile. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

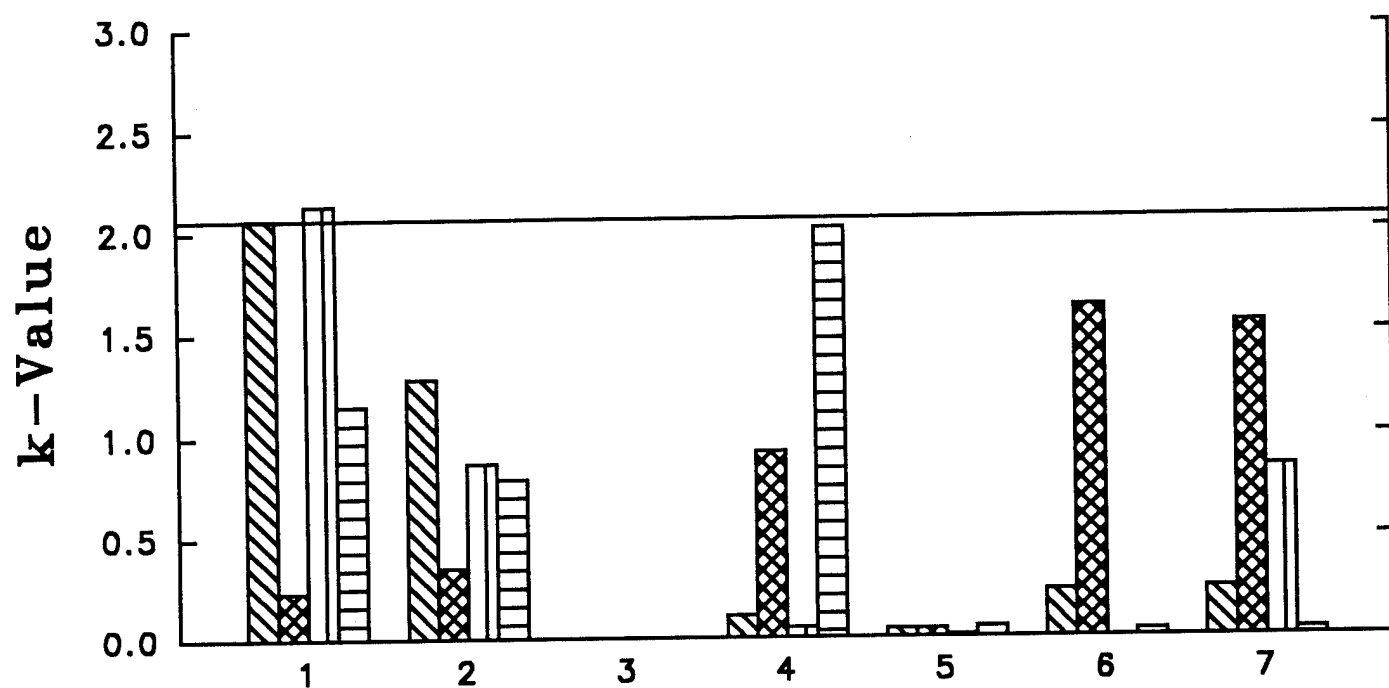
Figure 4. Intralaboratory k and interlaboratory h values for sodium acetate. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

Figure 5. Intralaboratory k and interlaboratory h values for sodium arsenate. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

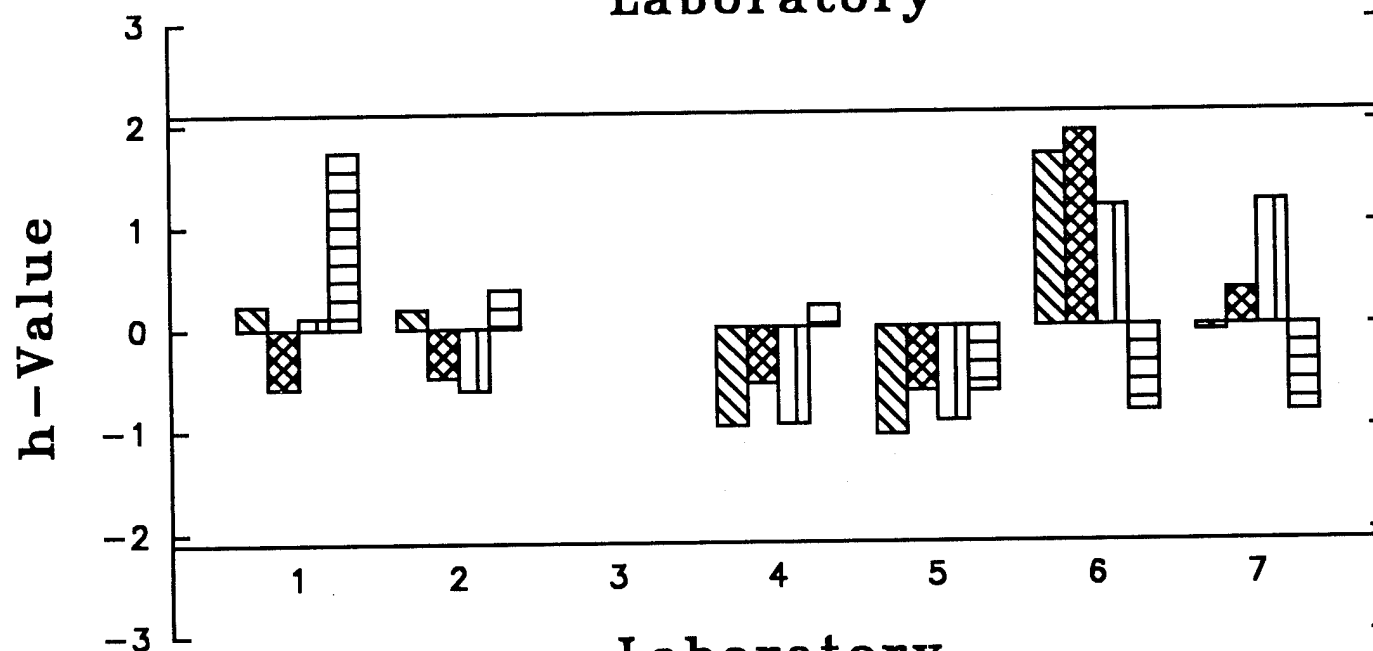
Figure 6. Intralaboratory k and interlaboratory h values for copper sulfate. (——) acceptable limit of variation. **A.** k values for intralaboratory variation. **B.** h values for interlaboratory variation.

p3a

Figure 1



Laboratory



Laboratory

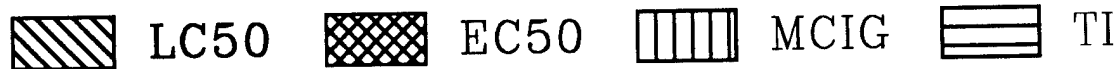


Figure 2

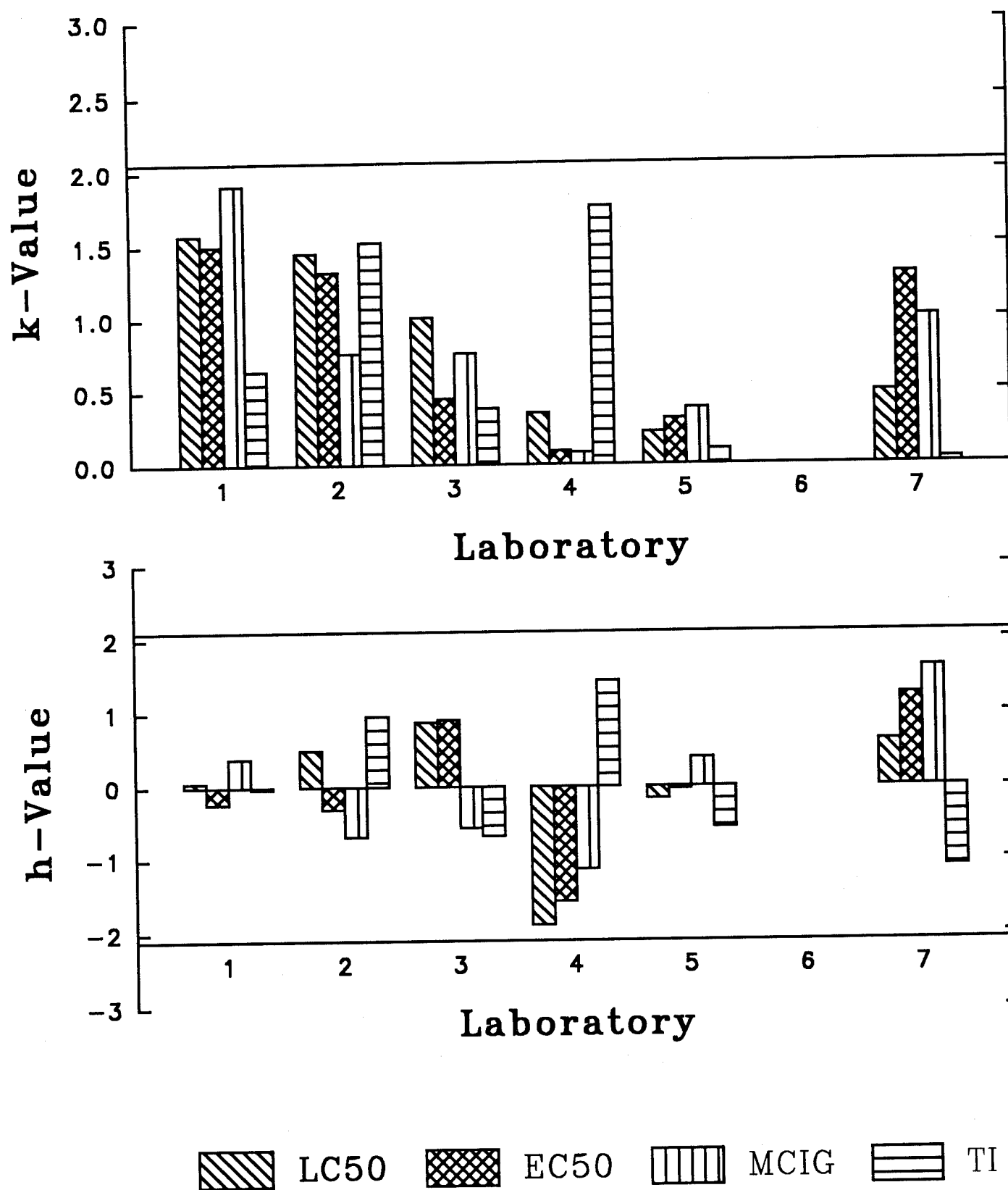


Figure 3

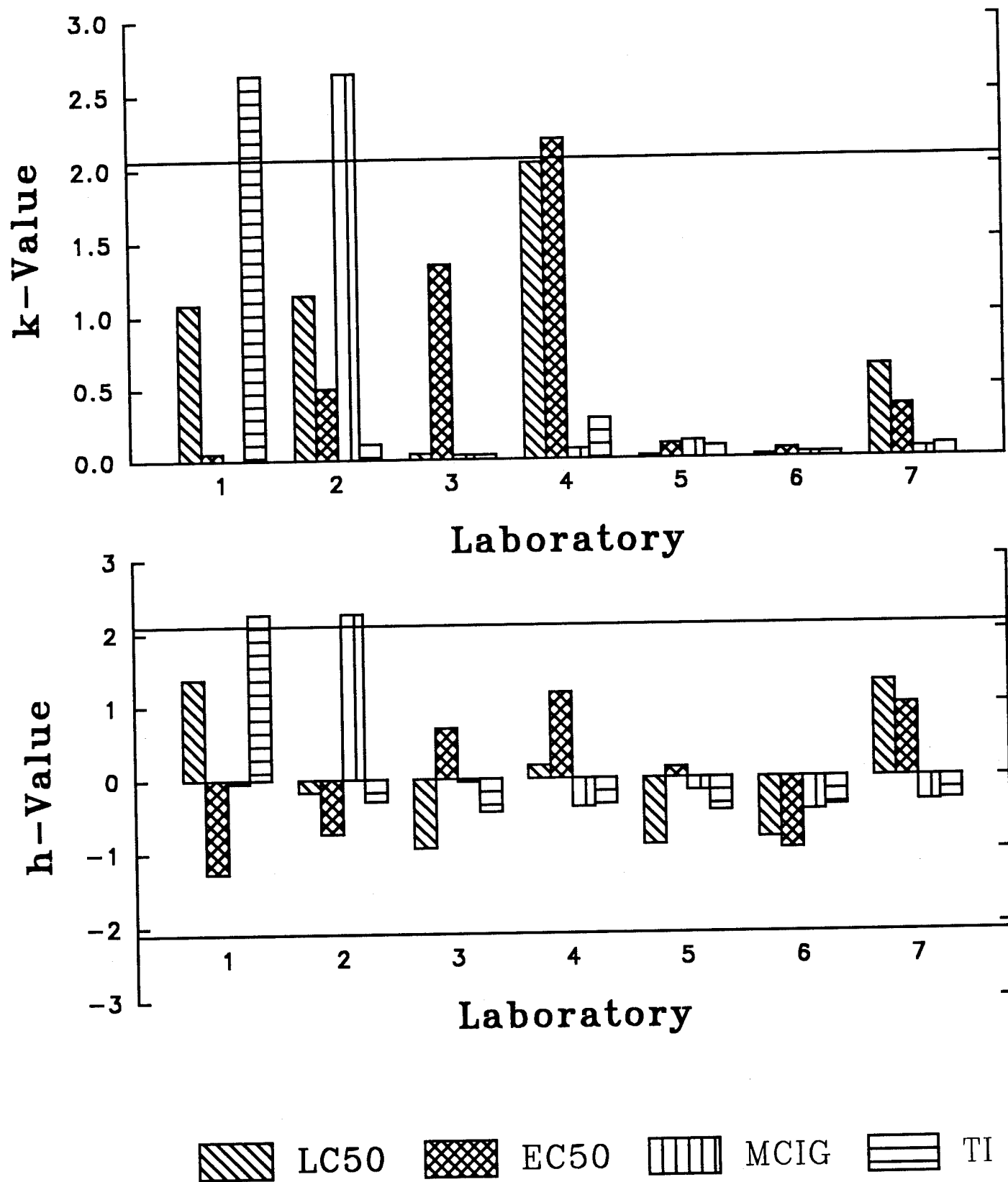


Figure 4

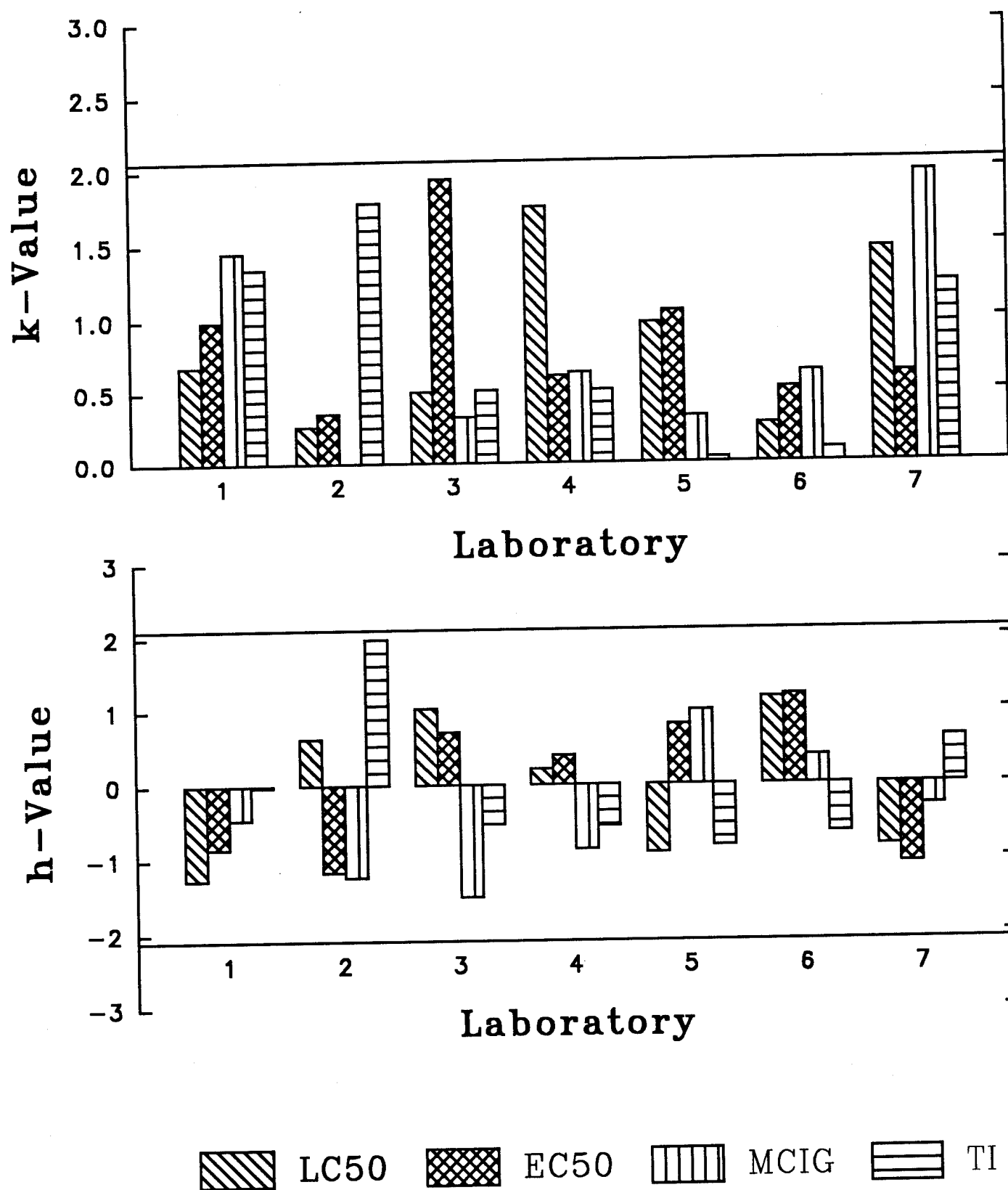


Figure 5

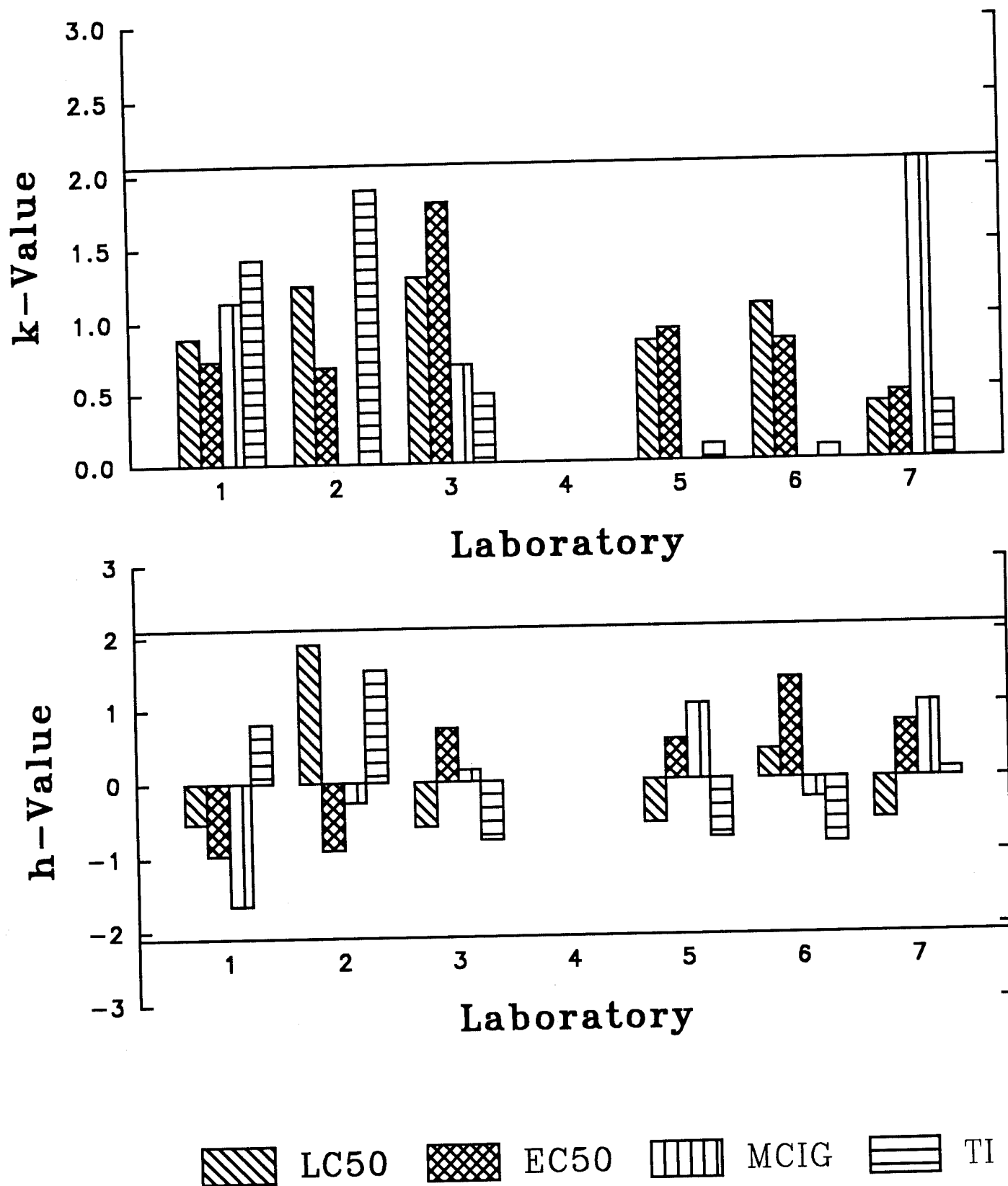
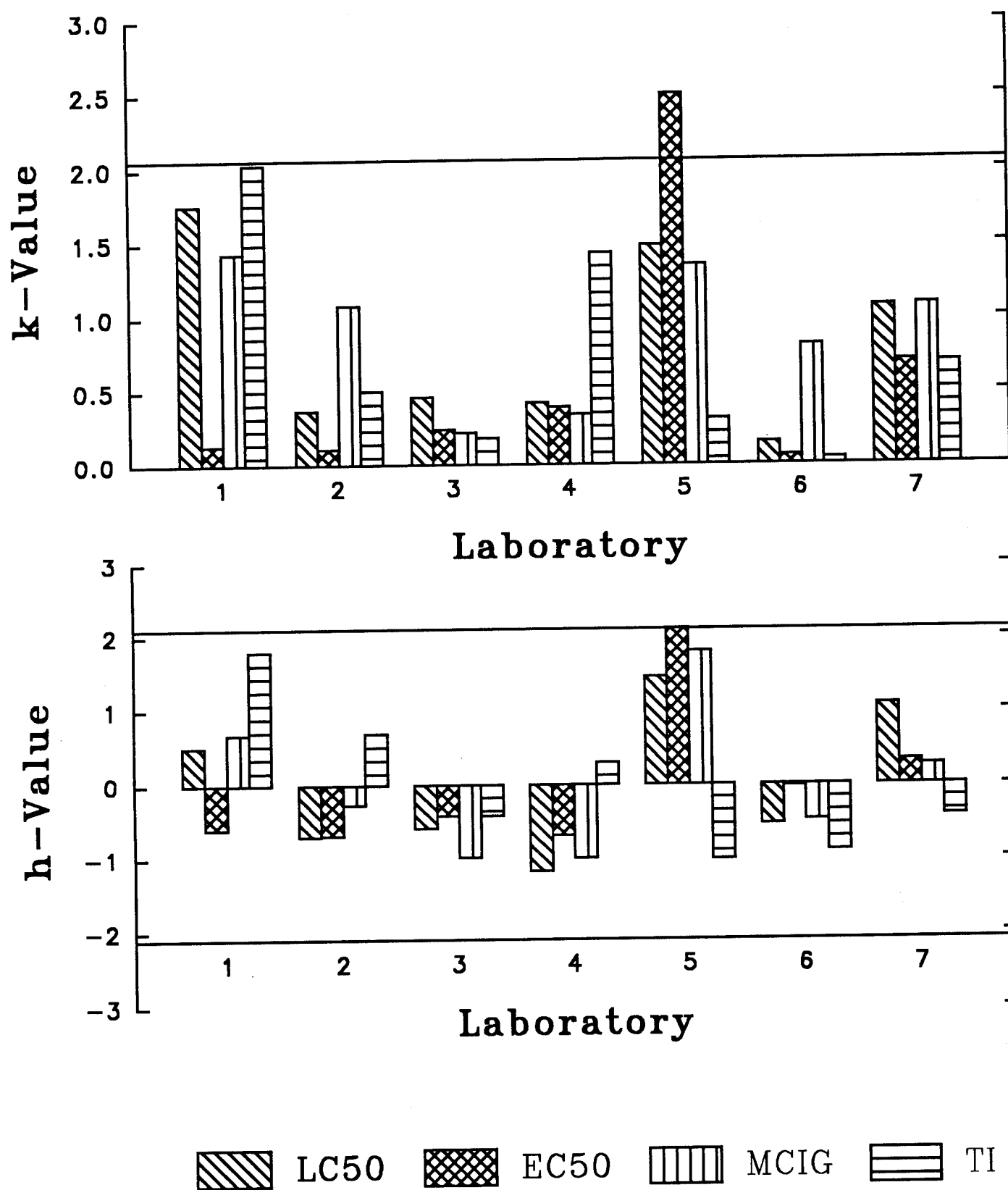


Figure 6



APPENDIX 1

INTERMEDIARY DATA TABLES AND FIRST PAGES FOR EACH COMPOUND

MONOSODIUM GLUTAMATE

PHASE III COMPOUND A DATA (P3A) MSG

FETAX ILS STUDY; 7 LABS, 8 LEVELS, 3 REPLICATES

PAGE 1

ORIGINAL DATA TABLE WITH MATERIALS

		LAB	LC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI	EC50	CILO	CIHI
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Table 1. Results of the Interlaboratory Study for Monosodium Glutamate.

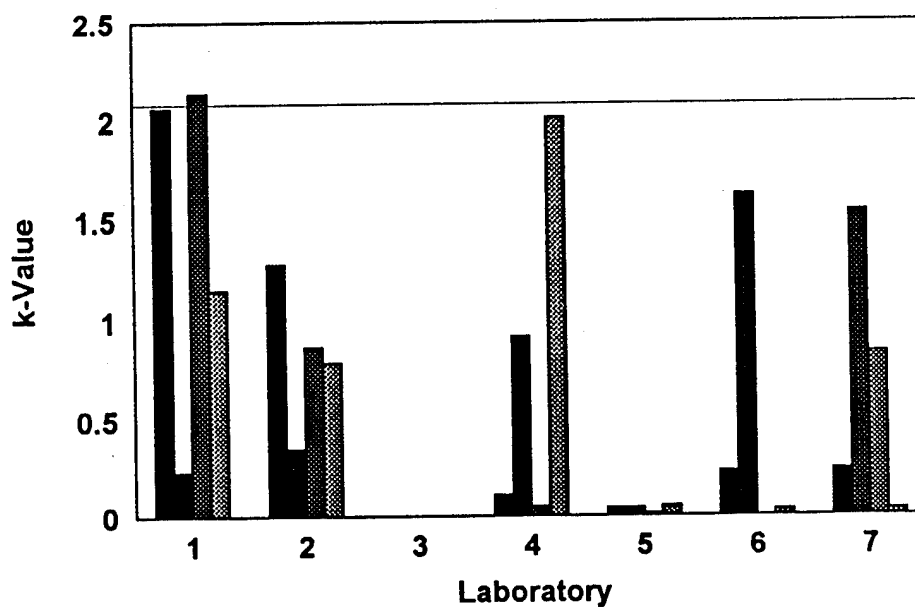
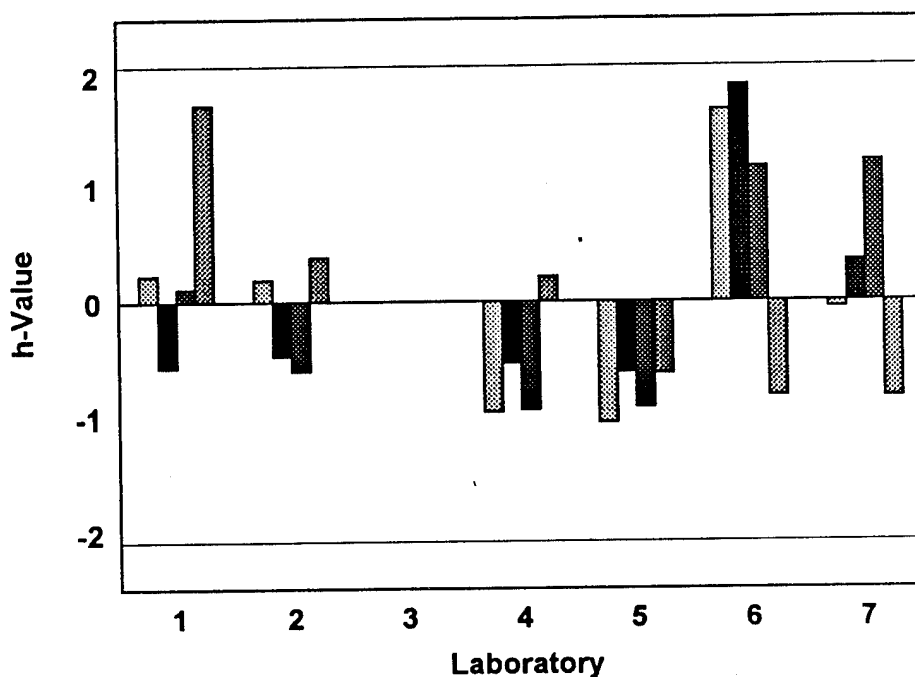
Laboratory	Replicate (n)	Mean		LC50		Mean		EC50		Mean		TI		Mean		MCIG ³		Mean		MCIG	
		LC50 (mg/ml)	CV1 (%)	LC50 (mg/ml)	CV1 (%)	EC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	EC50 (mg/ml)	CV (%)	CV (%)	CV (%)	TI ²	TI ²	MCIG ³ (mg/ml)	CV (%)	MCIG (mg/ml)	CV (%)	MCIG (mg/ml)	CV (%)
1	1	4.07		7.80		0.46		0.51		26.6		29.3		15.4		10.0		3.92		110.1	
	2	5.64			54.0	0.37										1.3					
	3	13.68				0.69										0.5					
2	1	8.02		7.56		1.32		1.02		20.5		41.1		7.4		4.0		1.50		117.9	
	2	4.15			34.6	0.86										0.3					
	3	10.50				0.89										0.3					
3	1																				
	2																				
	3																				
4	1	1.35		1.07		0.08		0.65		84.3		449.2		1.7		0.3		0.38		33.3	
	2	1.07			21.4	1.39										0.5					
	3	0.79				0.48															
5	1	0.66		0.56		0.26		0.24		9.7		8.2		2.3		0.4		0.42		5.7	
	2	0.48			13.4	0.21										0.5					
	3	0.54				0.26										0.4					
6	1	16.10		15.96		11.66		12.96		7.4		8.2		1.2		7.5		7.50		0.00	
	2	16.44			2.9	13.96										7.5					
	3	15.34				13.27										7.5					
7	1	5.47		6.17		3.90		5.19		17.7		11.3		1.2		10.0		7.67		22.2	
	2	6.52			8.0	5.67										7.0					
	3	6.51				5.99										6.0					

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of Monosodium Glutamate



FETAX Summary Sheet

Test No. DEF 1

Test Material	P3A	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	11-30-92
Composition/Purity		Test End Date	12-4-92
Solvent	NONE	Conc.	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.1	7.1	7	7.1	
Control		7.5	7.7	7.4	7.2
Highest Concentration		7.12	7.15	7	6.9

<div>No. Dead or Malformed</div> <div>----- X 100 = %</div> <div>Total Number</div>	Mortality Record		Malformation Record	
	1 : 100 X 100 = 1%		5 : 99 X 100 = 5.1%	
	C19 : E19 X 100 = G19		H19 : J19 X 100 = L19	
	Control Length 9.87		Solvent Control Length	
	Minimum Concentration to Inhibit Growth (MCIG)		10.00	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.1	T-test
LOEL	5	0.25	T-test
LC50	4.07	EC50	0.460
95% CL	2.88 -- 5.76	95% Confidence limits	0.303 ---- 0.698
Test Teratogenic Index (TI = LC50/EC50):		8.85	
95% Confidence limits		5.14 --	15.24

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 2

Test Material	P3A	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-7-92
Composition/Purity		Test End Date	12-11-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.2	7.3	7.2	7.2	
Control		7.6	7.4	7.5	7.3
Highest Concentration		7.5	7.7	7.8	7.8

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	7 : 100 X 100 = 7%	4 : 93 X 100 = 4.3%
Solvent Control	: X 100 =	: X 100 =
Control Length	C20	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	J21 1.25	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5	N.A.	T-test
LOEL	7.5	0.1	T-test
LC50	5.638	EC50	0.372
95% CL	5.055 -- 6.289	95% Confidence limits	0.241 ---- 0.576

Test Teratogenic Index (TI = LC50/EC50):	15.15
95% Confidence limits	9.66 -- 23.75

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 3

Test Material	P3A	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-13-92
Composition/Purity		Test End Date	12-17-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.2	7.1	7	7.1	
Control		7.6	7.4	7.6	7.3
Highest Concentration		7.3	7.4	7.3	7.2

No. Dead or Malformed				
	X 100 = %			
Total Number	Mortality Record		Malformation Record	
FETAX Control	5 : 100	X 100 = 5%	4 : 95	X 100 = 4.2%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	C20	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	J21 0.5			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.75	0.25	T-test
LOEL	5	0.5	T-test
LC50	13.676	EC50	0.694
95% CL	11.488 -- 16.279	95% Confidence limits	0.538 ---- 0.894
Test Teratogenic Index (TI = LC50/EC50):		19.71	
95% Confidence limits		14.49 -- 26.82	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 1

Test Material	Unknown P3A	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU
CAS No.	B5	Lot No.	E5
Composition/Purity	C6	Test Start Date:	Feb 1 1993
Solvent	B7	Test End Date	Feb 5 1993
Conc.	E7	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.0	7.2	7.0	7.0	X
Control	X	7.8	7.9	7.9	7.6
Highest Concentration	X	7.9	8.0	7.7	7.7

No. Dead or Malformed

----- X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length

Mortality Record		Malformation Record	
5 : 100	X 100 = 5%	6 : 95	X 100 = 6.3%
C19 : E19	X 100 = G19	H19 : J19	X 100 = L19

0.9

Solvent Control Length

J20

Minimum Concentration to Inhibit Growth (MCIG)

4 mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	N.A.	T-test
LOEL	8	0.25	T-test
LC50	8.018	EC50	1.3164
95% CL	7.332 -- 8.767	95% Confidence limits	1.0067 ---- 1.7214

Test Teratogenic Index (TI = LC50/EC50): 6.09

95% Confidence limits 4.59 -- 8.08

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 2

Test Material	Unknown P3A	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU
CAS No.	B5	Lot No.	E5
Test Start Date:	Feb 8 1993	Test End Date	Feb 12 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.0	7.1	7.2	X
Control	X	7.1	7.7	7.4	7.4
Highest Concentration	X	7.7	7.8	8.2	7.7

No. Dead or Malformed

_____ X 100 = %

MALFORMATION EXCEED ASTM LIMIT

Total Number	Mortality Record		Malformation Record	
FETAX Control	10 : 100	X 100 = 10%	7 : 90	X 100 = 7.8%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	0.9 cm	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.25 mg/ml			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	4	0.5	T-test
LOEL	6	0.75	T-test
LC50	4.148	EC50	0.861
95% CL	3.830 -- 4.494	95% Confidence limits	0.626 ---- 1.183

Test Teratogenic Index (TI = LC50/EC50): 4.82

95% Confidence limits 3.47 -- 6.69

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 3 *9/11/93*

Test Material	Unknown P3A	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU
CAS No.	B5	Lot No.	E5
Test Start Date:	Mar 1 1993	Test End Date	Mar 5 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.0	7.0	7.0	6.9	X
Control	X	7.0	7.2	7.4	7.6
Highest Concentration	X	7.5	7.6	7.7	7.8

No. Dead or Malformed	MALFORMATION EXCEED ASTM LIMIT			
_____ X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	7 : 100	X 100 = 7%	9 : 93	X 100 = 9.7%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	C20	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.25 0.25			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	1	T-test
LOEL	12	14	T-test
LC50	10.495	EC50	0.886
95% CL	7.994 -- 13.779	95% Confidence limits	0.237 ---- 3.321
Test Teratogenic Index (TI = LC50/EC50):		11.84	
95% Confidence limits		3.07 -- 45.61	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : F33 X 100 = G33	H33 : J33 X 100 = 13%
2500 mg/L	C34 : L34 X 100 = G34	H33 : J34 X 100 = 14%

CL= Confidence limits

FETAX Summary Sheet

Test No. ~~03~~ 4

Test Material	P3A	Investigator	J3
Source		Laboratory	J4
CAS No.	Lot No.	Test Start Date:	5/17/93
Composition/Purity		Test End Date	5/21/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	71	7.2	7.1	7	
Control		7.3	7.2	7.1	7.3
Highest Concentration		7.5	7.7	7.9	8.1

No. Dead or Malformed
X 100 = %
Total Number
FETAX Control
Solvent Control

MALFORMATION EXCEED ASTM LIMITS

Mortality Record	Malformation Record
2 : 100 X 100 = 2%	10 : 98 X 100 = 10.2%
: X 100 =	: X 100 =
Control Length (mm)	Solvent Control Length (mm)
Minimum Concentration to Inhibit Growth (MCIG)	mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.5	T-test
LOEL	2	5	T-test
LC50	1.354	EC50	0.076
95% CL	1.46E-07 -- 1.3E+07	95% Confidence limits	1E-10 ---- 6E+07
Test Teratogenic Index (TI = LC50/EC50):		17.73	
95% Confidence limits		9.35E-11 -- 3.36E+12	

Percent effect	LC	EC
5	3.1484E-05	2E-07
16	0.0021389	3E-05
50	1.35387185	0.076
84	856.966253	171.3
95	58218.6083	26615

FETAX Summary Sheet

Test No. 2

Test Material	P3A	Investigator	Gillett
Source		Laboratory	ERL-C
CAS No.	Lot No.	Test Start Date:	5/17/93
Composition/Purity		Test End Date	5/21/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	7.1	7.1	7.2	
Control		7.5	7.2	7.3	7.5
Highest Concentration		7.4	7.7	7.8	7.9

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm)

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

4 : 100

X 100 =

4%

6 : 96

X 100 =

6.3%

:

X 100 =

:

X 100 =

Solvent Control Length (mm)

0.25

mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	1	T-test
LOEL	2	2	T-test
LC50	1.069	EC50	1.386
95% CL	0.509	--	2.246
95% Confidence limits	0.525	----	3.661

Test Teratogenic Index (TI = LC50/EC50):

0.771

95% Confidence limits

0.227

--

2.618

Percent effect	LC	EC
5	0.1473788	0.138
16	0.3226661	0.343
50	1.0693508	1.386
84	3.543946	5.604
95	7.7589951	13.97

FETAX Summary Sheet

Test No.

3

Test Material	P3A	Investigator	Gillett
Source		Laboratory	ERL-C
CAS No.	Lot No.	Test Start Date:	5/16/93
Composition/Purity		Test End Date	5/20/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.2	7.2	7.1	
Control		7.4	7.3	7.3	7.6
Highest Concentration		7.2	7.4	8	8.1

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number

Mortality Record

Malformation Record

FETAX Control

2 : 100

X 100 =

2%

10 : 98

X 100 =

10.2%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm)

Solvent Control Length (mm)

Minimum Concentration to Inhibit Growth (MCIG)

0.5

mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.25	T-test
LOEL	1	0.5	T-test
LC50	0.790	EC50	0.482
95% CL	0.588 -- 1.063	95% Confidence limits	0.405 ---- 0.575

Test Teratogenic Index (TI = LC50/EC50):

1.638

95% Confidence limits

1.161

--

2.312

Percent effect	LC	EC
5	0.4188964	0.279
16	0.5383593	0.346
50	0.7901094	0.482
84	1.1595839	0.672
95	1.4902799	0.835

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>UNKNOWN P3A</u>	Investigator <u>TURLEY</u>
Source <u>OSU</u>	Lab <u>UMD-WREC</u>
CAS No. <u>-</u> Lot No. <u>-</u>	Test Start Date <u>NOVEMBER 17, 1992</u>
Composition/Purity <u>-</u>	Test End Date <u>NOVEMBER 21, 1992</u>
Solvent <u>-</u> Conc. <u>-</u>	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	7.20	7.22	7.17	7.24	7.26
Control	7.80	7.72	7.84	7.76	7.85
Highest Conc.	7.35	7.31	7.37	7.35	7.39

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>1</u> : <u>100</u> X 100 = <u>1</u> %	<u>8</u> : <u>99</u> X 100 = <u>8</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length <u>10.09</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.45</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	0.10	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	0.15	Bonferroni T-test
LC ₅₀ <u>0.48</u> mg/ml (Trimmed Spearman-Kärber)	EC ₅₀ <u>0.21</u> mg/ml (Trimmed Spearman-Kärber)		
95% Confidence limits <u>0.42-0.56</u>	95% Confidence Limits <u>0.16-0.27</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 2.29

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>UNKNOWN P3A</u>		Investigator <u>TURLEY</u>
Source <u>0.5U</u>		Lab <u>UMD-WREC</u>
CAS No.	Lot No.	Test Start Date <u>NOV. 30</u>
Composition/Purity		Test End Date <u>DEC. 3, 1992</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock	7.19	7.16	7.21	7.22	7.19
Control	7.79	7.84	7.90	7.82	7.86
Highest Conc.	7.30	7.29	7.36	7.31	7.36

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>2</u> : <u>100</u> X 100 = <u>2</u> %	<u>8</u> : <u>98</u> X 100 = <u>8</u> %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length <u>10.13</u> mm	Solvent Control Length ___ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.40</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀ <u>0.54</u> mg/ml Trimmed Spearman-Kärber	EC ₅₀ <u>0.26</u> mg/ml Trimmed Spearman-Kärber		
95% Confidence limits <u>0.50 - 0.59</u> mg/ml	95% Confidence Limits <u>0.20 - 0.32</u> mg/ml		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 2.08

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX SUMMARY SHEET

Test No. 4

Test Material <u>UNKNOWN P3A</u>		Investigator <u>TURLEY</u>
Source <u>O.S.U</u>		Lab <u>UMD-WREC</u>
CAS No.	Lot No.	Test Start Date <u>12/4/92</u>
Composition/Purity		Test End Date <u>12/8/92</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	7.13	7.16	7.19	7.14	7.18
Control	7.65	7.63	7.61	7.64	7.69
Highest Conc.	7.21	7.18	7.26	7.24	7.28

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number	$\frac{2}{100} \times 100 = 2\%$	$\frac{7}{98} \times 100 = 7\%$
Solvent Control	$\frac{-}{-} \times 100 = -\%$	$\frac{-}{-} \times 100 = -\%$
Control Length <u>10.03</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.40</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀ <u>0.66</u> mg/ml (Trimmed Spearman-Kärber)	EC ₅₀ <u>0.26</u> mg/ml (Trimmed Spearman-Kärber)		
95% Confidence limits <u>0.60-0.72</u>	95% Confidence Limits <u>0.20-0.32</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 2.54

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX SUMMARY SHEET

Test Material <u>P3A</u>		Investigator <u>D.A. Jansen</u>
Source		Lab <u>WT/CVM</u>
CAS No.	Lot No.	Test Start Date <u>11/30/92</u>
Composition/Purity		Test End Date <u>12/4/92</u>
Solvent	Conc.	Test Units (l.o., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.10	7.10	7.13	7.13	—
Control	7.83	7.69	7.58	7.43	7.37
Highest Conc.	—	7.62	7.74	7.96 ^{de}	8.19 ^{de}

FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed	X 100 = %				
Total Number					
Solvent Control		0 : 100 X 100 = 0 %		2 : 100 X 100 = 2 %	
Control Length mm		: X 100 = %		: X 100 = %	
Minimum Concentration to Inhibit Growth (MCIG)		Solvent Control Length mm			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	15 both	< 7.5 both	
LOEL	17.5 both	7.5 both	
LC ₅₀	16.10	EPA EC ₅₀ 11.66	
95% Confidence limits	(15.8-16.5) / Prob. t	95% Confidence Limits (9.0-15.2) > L ₁ W	

TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)	1.38 - L ₁ W / EC ₅₀
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POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <u>not</u>	: X 100 = %	: X 100 = %
2500 mg/L <u>done</u>	: X 100 = %	: X 100 = %

D.A.J.

12/14/92

EPA
Prob. t
12.39
(11.8-13.1)
TSK
12.1
(11.8-13.1)

FETAX SUMMARY SHEET

Test No. 2

Test Material	P3A	Investigator	D.A. JAWSON
Source		Lab	WT/CVY
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/8/92
Solvent		Test End Date	12/12/92
Conc.		Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.11	7.11	7.10	7.10	—
Control	7.87	7.80	7.62	7.62	7.62
Highest Conc.	—	7.64	7.77	7.95 ^{ac}	8.02 ^{ac}

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed	X 100 = %		
Total Number		0 : 100 X 100 = 0 %	3 : 100 X 100 = 3 %
Solvent Control		_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	—	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 7.5			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	15.0 both	100 both	
LOEL	175 both	125 both	
LC ₅₀	16.44 TSK	EC ₅₀ 13.96 CFW	
95% Confidence limits	16.2-16.6	95% Confidence Limits (12.6 15.5)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

1.18

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

D.A.D.

12/14/92

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>P3A</u>		Investigator <u>J. A. Dawson</u>
Source		Lab <u>WICUM</u>
CAS No.	Lot No.	Test Start Date <u>11/4/93</u>
Composition/Purity		Test End Date <u>1/8/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock	7.12	7.10	7.12	7.07	—
Control	7.77	7.68	7.54	7.56	7.51
Highest Conc.	—	7.63	7.74	7.78	7.81

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u> </u> X 100 = %		
Total Number	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>7.5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	12.5 <u>bdh</u>	—	homogeneity failed for EC ₅₀ - NOEL-LOEL
LOEL	15.0	—	
LC ₅₀ 15.34	<u>> 73K</u>		EC ₅₀ 13.27
95% Confidence limits (15.0 - 15.7)			95% Confidence Limits (12.9 - 13.6) <u>> 73K</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.16

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <u>not done</u>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L <u>done</u>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

J. A. Dawson
1/8/93

FETAX SUMMARY SHEET

Test No. 1

Test Material	P3A	Investigator	Fort
Source	ILS, Phase 3	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/14/92
Solvent		Conc.	
		Test End Date	12/18/92
		Test Units (i.e., mg/ml)	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.3	7.2	7.3	7.4	
Control	7.9	7.9	7.8	7.9	
Highest Conc.	7.6	7.5	7.5	7.6	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
	$\frac{\quad}{\quad} \times 100 = \quad \%$	$\frac{\quad}{\quad} \times 100 = \quad \%$
Solvent Control	$\frac{\quad}{\quad} \times 100 = \quad \%$	$\frac{\quad}{\quad} \times 100 = \quad \%$
Control Length 97.3 mm	Solvent Control Length	mm
Minimum Concentration to Inhibit Growth (MCIG)	10.0	

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.1	0.1	Williams
LOEL	1.0	1.0	Williams
LC ₅₀	5.47	EC ₅₀	3.90
95% Confidence Limits 4.64 - 6.46		95% Confidence Limits 3.08 - 4.93	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 1.40

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	$\frac{\quad}{\quad} \times 100 = \quad \%$	$\frac{\quad}{\quad} \times 100 = \quad \%$
2500 mg/L	$\frac{\quad}{\quad} \times 100 = \quad \%$	$\frac{\quad}{\quad} \times 100 = \quad \%$

FETAX SUMMARY SHEET

Test Material P3A		Investigator Fort
Source ILS		Lab SBL
CAS No.	Lot No.	Test Start Date 2/5/93
Composition/Purity		Test End Date 2/9/93
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.3	7.4	7.4	7.4	
Control	8.0	7.9	7.9	7.9	
Highest Conc.	7.5	7.5	7.6	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = % Total Number	0 : 100 X 100 = 0 %	0 : 100 X 100 = 0 %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length 97.0 mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) 7.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	4.0	4.0	Williams Test
LOEL	5.0	3.0	
LC ₅₀	6.52	EC ₅₀	5.67
95% Confidence limits 6.25 - 6.80		95% Confidence Limits 5.37 - 5.99	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | **1.15**

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3A	Investigator	Fort
Source	ILS	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	2/6/93
Solvent		Conc.	
		Test End Date	2/10/93
		Test Units (i.e., mg/ml)	mg/ml

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.4	7.5	7.5	7.5	
Control	8.0	7.9	7.9	7.8	
Highest Conc.	7.6	7.6	7.6	7.6	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	0 : 100 X 100 = 0 %	0 : 100 X 100 = 0 %
Solvent Control	: X 100 = %	: X 100 = %
Control Length 96.0 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 6.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	5.0	4.0	Williams Test
LOEL	6.0	5.0	
LC ₅₀	6.51	EC ₅₀	5.99
95% Confidence limits	6.32-6.72	95% Confidence Limits	5.69-6.30

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 1.09

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

ASCORBIC ACID

PHASE III COMPOUND B DATA (P3B) ASCORBIC ACID

FETAX ILS STUDY; 7 LABS, 8 LEVELS, 3 REPLICATES

PAGE 1

units are in mg/ml

ORIGINAL DATA TABLE WITH MATERIALS

		SUBTOTALS:															STD:	CV:				
		LC50	CILO	CIHI	EC50	CILO	CIHI	MCIG	TI	LOEC/MORT	LOEC/MALF	J			LC50	EC50	MCIG	TI	STD:	CV:		
BANTLE/ RAYBURN	1	11.04	9.97	12.23	7.01	6.18	7.96	5.00	1.6	12.00	7.50	10.98	3.238	29.5								
	2	6.98	6.05	8.04	5.31	4.48	6.30	10.00	1.3	8.00	6.00	6.60	0.932	14.1								
	3	14.91	14.08	15.79	7.48	6.79	8.24	10.00	2.0	18.00	8.00	8.33	2.357	28.3								
	TI																			1.66	0.280	16.8
BANTLE/ HULL	1	15.21	14.26	16.22	7.00	6.25	7.85	2.00	2.2	16.00	6.00	13.06	2.975	22.8								
	2	8.85	7.49	10.47	6.69	5.98	7.49	2.00	1.3	8.00	8.00	6.29	0.800	12.7								
	3	15.11	13.23	17.26	5.17	3.95	6.77	4.00	2.9	12.00	2.00	2.67	0.943	35.4								
	TI																			2.08	0.654	31.5
FINCH	1	15.61	15.02	16.22	11.33	10.67		2.00	1.4	16.00	12.00	14.83	2.067	13.9								
	2	16.88	15.50	18.38	11.99	11.05	13.01	4.00	1.4	18.00	14.00	11.60	0.281	2.4								
	3	12.00	7.70	18.70	11.49	9.67	13.66	4.00	1.0	18.00	20.00	3.33	0.943	28.3								
	TI																			1.28	0.165	12.9
LINDER/ BUCHWALTER	1	2.78	2.24	3.45	0.82	0.69	0.97	0.30	3.4	5.00	1.00	1.82	0.717	39.5								
	2	1.61	1.26	2.04	0.70	0.48	1.03	0.30	2.3	1.00	0.50	0.74	0.057	7.6								
	3	1.06	0.93	1.22	0.70	0.51	0.96	0.50	1.5	1.00	0.50	0.37	0.094	25.7								
	TI																			2.45	0.769	31.3
BURTON/ TURLEY	1	9.26	8.93	9.61	7.24	6.66	7.86	9.00	1.3			9.91	0.458	4.6								
	2	10.21	9.79	10.66	7.69	7.10	8.33	8.00	1.3			7.43	0.192	2.6								
	3	10.25	9.65	10.90	7.35	6.69	8.07	8.00	1.4			8.33	0.471	5.7								
	TI																			1.33	0.047	3.6
DAWSON	1											#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
	2											#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
	3											#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
STOVER/ FORT	1	12.20	11.33	13.13	11.94	10.59	13.46	15.00	1.0	10.00	10.00	13.61	0.996	7.3								
	2	14.26	14.00	14.54	13.47	13.14	1.80	16.00	1.1	12.00	12.00	13.03	0.775	6.0								
	3	14.36	14.08	14.65	13.68	13.43	13.92	13.00	1.0	13.00	13.00	14.67	1.247	8.5								
	TI																			1.04	0.016	1.5
GRAND TOTAL		STD:															CV:					
LC50		10.70	4.76	44.5																13.61	0.996	7.3
EC50		7.61	4.0322	53.0																13.03	0.775	6.0
MCIG		6.28	4.91	78.1																14.67	1.247	8.5
TI		1.41	0.65	46.3																1.04	0.016	1.5
LOEC (mort)		11.20	5.53	49.4																		
LOEC (malif)		8.07	5.64692	70.0																		

Table 2. Results of the Interlaboratory Study for Ascorbic Acid.

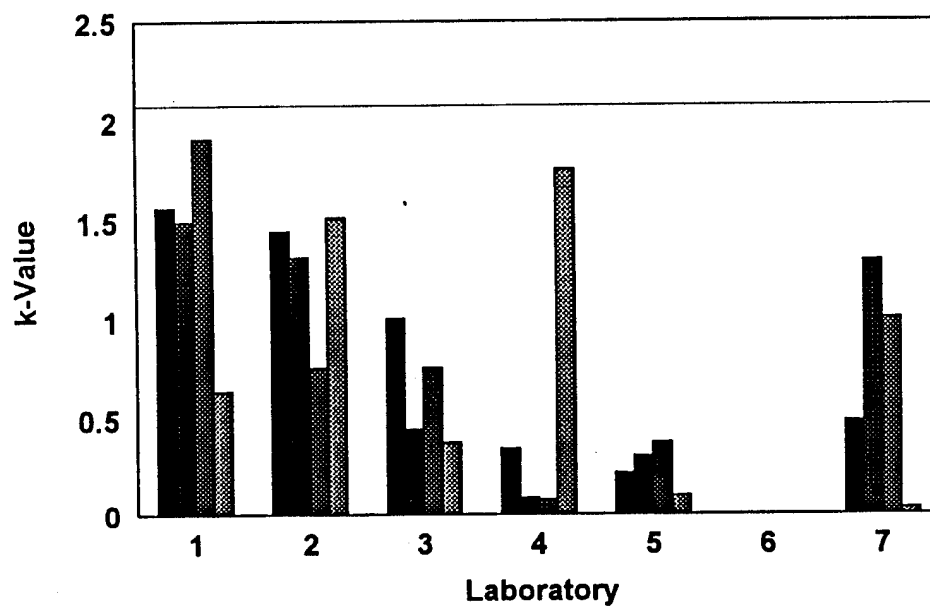
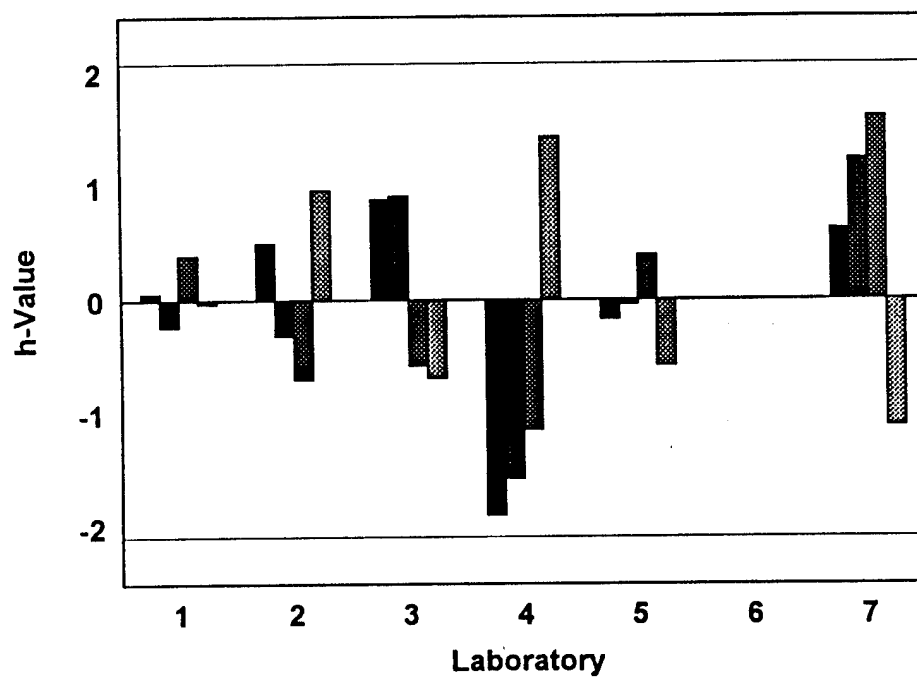
Laboratory	Replicate (n)	Mean LC50			Mean EC50			Mean TI			Mean MCIG		
		LC50 (mg/ml)	LC50 (mg/ml)	CV ¹ (%)	EC50 (mg/ml)	EC50 (mg/ml)	CV (%)	TI ² (%)	TI ² (%)	CV (%)	MCIG ³ (mg/ml)	MCIG (mg/ml)	CV (%)
1	1	11.04			7.01						5.0		
	2	6.98	10.98	29.5	5.31	6.60	14.1	1.7	16.8		10.0	8.33	28.3
	3	14.91			7.48						10.0		
2	1	15.21			7.00						2.0		
	2	8.85	13.06	22.8	6.69	6.29	12.7	2.1	31.5		2.0	2.67	35.4
	3	15.11			5.17						4.0		
3	1	15.61			11.33						2.0		
	2	16.88	14.83	13.9	11.99	11.60	2.4	1.3	12.9		4.0	3.33	28.3
	3	12.00			11.49						4.0		
4	1	2.78			0.82						0.3		
	2	1.61	1.82	39.5	0.70	0.74	7.6	2.5	31.3		0.3	0.37	25.7
	3	1.06			0.70						0.5		
5	1	9.26			7.24						9.0		
	2	10.21	9.91	4.6	7.69	7.43	2.6	1.3	3.6		8.0	8.33	5.7
	3	10.25			7.35						8.0		
6	1												
	2												
	3												
7	1	12.20			11.94						15.0		
	2	14.26	13.61	7.3	13.47	13.03	6.0	1.0	1.5		16.0	14.67	8.5
	3	14.36			13.68						13.0		

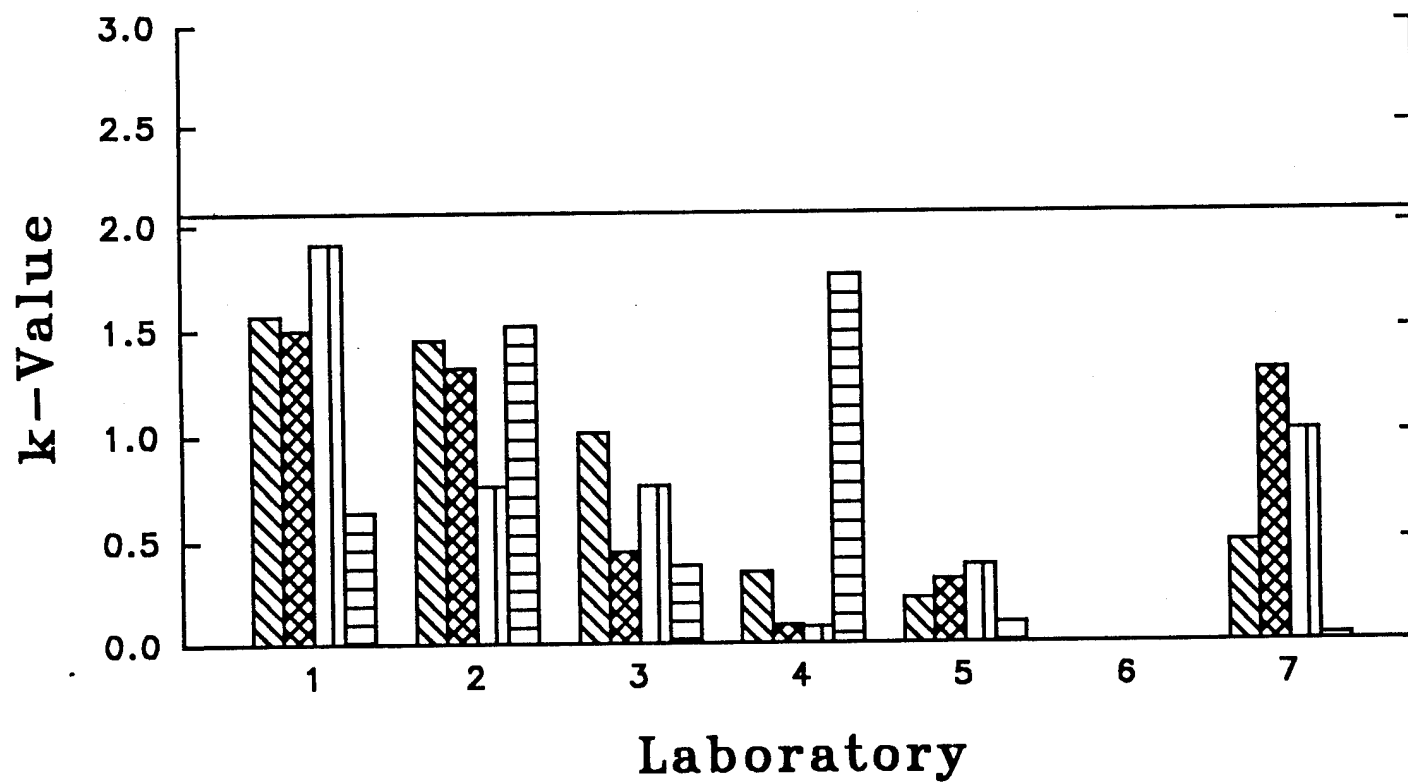
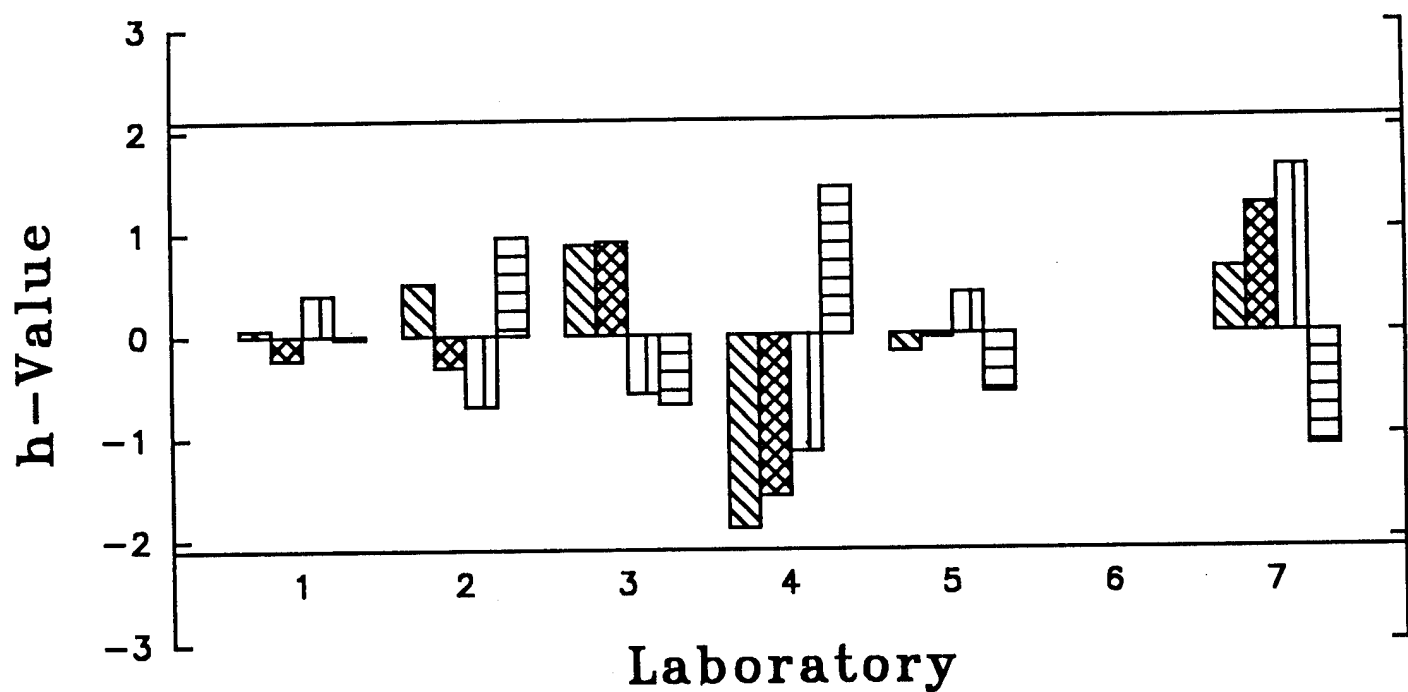
¹CV=Coefficient of Variation.


²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of Ascorbic Acid





 LC50
  EC50
  MCIG
  TI

FETAX Summary Sheet

Test No. DEF 1

Test Material	P3B	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	11-30-92
Composition/Purity		Test End Date	12-4-92
Solvent	NONE	Conc.	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	8.1	8	8.2	7.5	
Control		7.2	7.5	7.2	7
Highest Concentration		7.57	7.51	7.3	7.6

No. Dead or Malformed		
----- X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control	1 : 100 X 100 = 1%	5 : 99 X 100 = 5.1%
Control Length	C20	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	5.0	J21

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	5	T-test
LOEL	12	7.5	T-test
LC50	11.038	EC50	7.014
95% CL	9.967 -- 12.225	95% Confidence limits	6.179 ---- 7.961

Test Teratogenic Index (TI = LC50/EC50):	1.57
95% Confidence limits	1.34 -- 1.85

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 2

Test Material	P3B	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-7-92
Composition/Purity		Test End Date	12-11-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.5	7.2	7.6	7.4	
Control		7.4	7.3	7.6	7.3
Highest Concentration		7.5	7.3	7.3	7.3

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	10 : 100 X 100 = 10%	6 : 90 X 100 = 6.7%
Solvent Control	: X 100 =	: X 100 =
Control Length	C20	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	10	J21

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	4	T-test
LOEL	8	6	T-test
LC50	6.975	EC50	5.314
95% CL	6.052 -- 8.039	95% Confidence limits	4.483 ---- 6.300

Test Teratogenic Index (TI = LC50/EC50):	1.31
95% Confidence limits	1.05 -- 1.64

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 3

Test Material	P3B	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-13-92
Composition/Purity		Test End Date	12-17-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.9	7.2	8.3	7.7	
Control		7.3	7.5	7.3	7
Highest Concentration		7.2	7.4	7.1	7

No. Dead or Malformed		
	X 100 = %	
Total Number		
FETAX Control	Mortality Record	Malformation Record
	4 : 100 X 100 = 4%	4 : 96 X 100 = 4.2%
Solvent Control	C19 : E19 X 100 = G19	H19 : J19 X 100 = L19
Control Length	C20	Solvent Control Length J20
Minimum Concentration to Inhibit Growth (MCIG)	/ O J21	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	6	T-test
LOEL	18	8	T-test
LC50	14.907	EC50	7.475
95% CL	14.078 -- 15.785	95% Confidence limits	6.786 ---- 8.235

Test Teratogenic Index (TI = LC50/EC50):	1.99
95% Confidence limits	1.78 -- 2.23

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 1

Test Material	Unknown ^{P3P} 59A	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU <i>MB</i>
CAS No.	B5	Lot No.	E5
Test Start Date:	Feb 1 1993	Test End Date	Feb 5 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	8.0	8.0	7.7	7.9	X
Control	X	7.7	7.4	7.5	7.6
Highest Concentration	X	7.9	8.0	7.7	7.4

No. Dead or Malformed

_____ X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length

Mortality Record

9 : 100 X 100 = 9%

C19 : E19 X 100 = G19

Solvent Control Length

Malformation Record

6 : 91 X 100 = 6.6%

H19 : J19 X 100 = L19

J20

Minimum Concentration to Inhibit Growth (MCIG)

2 mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	14	4	T-test
LOEL	16	6	T-test
LC50	15.209	EC50	7.004
95% CL	14.264 -- 16.217	95% Confidence limits	6.252 ---- 7.846
Test Teratogenic Index (TI = LC50/EC50):			2.17
95% Confidence limits			1.91 -- 2.47

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 2

Test Material	Unknown P3B	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU <i>PHB</i>
CAS No.	B5	Lot No.	E5
Test Start Date:	Feb 8 1993	Test End Date	Feb 12 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.9	7.9	8.0	7.9	X
Control	X	7.7	7.4	7.5	7.4
Highest Concentration	X	7.2	7.0	6.8	6.9

No. Dead or Malformed	X 100 = %		MALFORMATION EXCEED ASTM LIM	
Total Number	Mortality Record		Malformation Record	
FETAX Control	10 : 100	X 100 = 10%	9 : 90	X 100 = 10.0%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	0.9 cm	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	2 mg/ml			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	7	7	T-test
LOEL	8	8	T-test
LC50	8.854	EC50	6.692
95% CL	7.487 -- 10.472	95% Confidence limits	5.982 ---- 7.487
Test Teratogenic Index (TI = LC50/EC50):		1.32	
95% Confidence limits		1.08 -- 1.62	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 3

Test Material	Unknown P3B	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU <i>JMB</i>
CAS No.	Lot No.	Test Start Date:	Mar 1 1993
Composition/Purity		Test End Date	Mar 5 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.8	7.9	8.0	8.1	X
Control	X	7.4	7.2	7.5	7.8
Highest Concentration	X	6.8	6.8	6.5	6.7

No. Dead or Malformed							MALFORMATION EXCEED ASTM LIM			
_____ X 100 = %										
Total Number	Mortality Record				Malformation Record					
FETAX Control	3	:	100	X 100 =	3%	10	:	97	X 100 =	10
Solvent Control	:			X 100 =		:			X 100 =	
Control Length	1	cm	Solvent Control Length							
Minimum Concentration to Inhibit Growth (MCIG)					4 mg/ml					

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	N.A.	T-test
LOEL	12	2	T-test
LC50	15.114	EC50	5.172
95% CL	13.234 -- 17.260	95% Confidence limits	3.949 ---- 6.772
Test Teratogenic Index (TI = LC50/EC50):		2.92	
95% Confidence limits		2.16 -- 3.95	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	: X 100 =	: X 100 =
2500 mg/L	: X 100 =	: X 100 =

CL= Confidence limits

FETAX Summary Sheet

Test No. 3-P3B

Test Material	P3B	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date	10 MAY 93
Solvent	-	Test End Date	14 MAY 93
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.9	7.0	7.5	7.0	
Control		7.9	7.9	7.9	7.9
Highest Concentration		7.5	7.4	7.7	7.2

No. Dead or Malformed		
..... X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control		
Control Length	9.641	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	2.0 ✓	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	14	10	T-test
LOEL	16	12	T-test
LC50	15.608 ✓	EC50	11.331 ✓
95% CL	15.019 -- 16.220	95% Confidence limits	10.673 ---- *****
Test Teratogenic Index (TI = LC50/EC50):		1.38 ✓	
95% Confidence limits		1.28 -- 1.48	

CL= Confidence limits

FETAX Summary Sheet

Test No. 5-P3B

Test Material	P3B	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	Lot No.	Test Start Date:	17 MAY 93
Composition/Purity		Test End Date	21 MAY 93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.5	7.0	7.2	7.2	
Control		7.9	7.9	7.9	7.9
Highest Concentration		7.8	7.3	7.7	7.3

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length

10.295

Mortality Record

1 : 100

X 100 =

1%

Malformation Record

5 : 99

X 100 =

5.1%

Solvent Control Length

Minimum Concentration to Inhibit Growth (MCIG)

4.0

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	16	8	T-test
LOEL	18	14	T-test
LC50	16.876	EC50	11.992
95% CL	15.497	95% Confidence limits	11.054 --- 13.009
Test Teratogenic Index (TI = LC50/EC50):			1.41
95% Confidence limits			1.25 -- 1.58

CL= Confidence limits

FETAX Summary Sheet

Test Material		Investigator	
Source		Laboratory	
CAS No.	Lot No.	Test Start Date:	
Composition/Purity		Test End Date	
Solvent	Conc.	Test Units (i.e., mg/ml)	

pH		Day 0	Day 1	Day 2	Day 3	Day 4
	Stock	E11	F11	G11	J11	
	Control		F12	G12	J11	K12
	Highest Concentration		F13	G13	J11	K13

No. Dead or Malformed		X 100 = %	
Total Number			
FETAX Control			
Solvent Control			
Control Length			
Minimum Concentration to Inhibit Growth (MCIG)			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	16	18	T-test
LOEL	18	20	T-test
LC50	11.99629	EC50	11.49150
95% CL	7.69582 -- 18.69988	95% Confidence limits	9.67021 -- 13.65583
Test Teratogenic Index (TI = LC50/EC50):		1.04	
95% Confidence limits		0.65 -- 1.68	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX SUMMARY SHEET

Test Material: P3B		Test No. Def #1
Source: Bantle		Investigator: Gillett
CAS No. — Lot No. —	Laboratory: CERL	
Composition / Purity: —		Test Start Date: 06/28/93
Solvent: N/A Conc. —		Test End Date: 07/02/93
		Test Units: (i.e., mg/ml) mg/ml

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.2	7.1	7.1	7.2	X
Control	X	7.5	7.4	7.4	7.6
Highest Conc.	X	6.9	7.1	7.5	7.2

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	<u>6</u> : <u>100</u> X 100 = <u>6</u> %	<u>4</u> : <u>94</u> X 100 = <u>4.3</u> %
Solvent Control: N.A.	— : — X 100 = — %	— : — X 100 = — %
Control Length: 0.907 ± 0.045 mm	Solvent Control Length: _____ mm	
Minimum Concentration to Inhibit Growth (MCIG): 0.3 mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.0 mg/ml	0.7 mg/ml	Williams
LOEL	5.0 mg/ml	1.0 mg/ml	Williams
LC ₅₀	2.78 mg/ml	EC ₅₀	0.82 mg/ml
95% Confidence Limits 2.24-3.45		95% Confidence Limits 0.69-0.97 mg/ml	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) = 3.39

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	— : — X 100 = — %	— : — X 100 = — %
2.5 mg/ml	— : — X 100 = — %	— : — X 100 = — %

FETAX SUMMARY SHEET

Test Material: P3B		Test No. Def. #2
Source: Bantle		Investigator: Gillett
CAS No. — Lot No. —	Laboratory: Corvallis Env. Res. Lab	
Composition / Purity: —		Test Start Date: 8/2/93
Solvent: — Conc. —		Test End Date:
		Test Units: (i.e., mg/ml)

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	7.1	7.3	7.4	X
Control	X	7.0	7.4	7.1	7.2
Highest Conc.	X	6.9	7.1	7.2	7.1

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number		
Solvent Control: NA 0.83 ± 0.74	8 : 100 X 100 = 8 %	10 : 92 X 100 = 10.8 %
Control Length: 0.616 ± 0.074 mm	— : — X 100 = — %	— : — X 100 = — %
Solvent Control Length: mm		
Minimum Concentration to Inhibit Growth (MCIG): 0.3		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.7 mg/ml	0.3 mg/ml	Williams
LOEL	1.0 mg/ml	0.5 mg/ml	Williams
LC ₅₀ 1.61		EC ₅₀ 0.70	
95% Confidence Limits 1.26-2.04 mg/ml		95% Confidence Limits 0.48-1.03 mg/ml	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) = 2.3

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	— : — X 100 = — %	— : — X 100 = — %
2.5 mg/ml	— : — X 100 = — %	— : — X 100 = — %

FETAX SUMMARY SHEET

Test Material: <u>P3B</u>		Test No. <u>#3</u> DEF.
Source: <u>Bottle</u>		Investigator: <u>Gille tt</u>
CAS No. <u> </u> Lot No. <u> </u>	Laboratory: <u>CERL</u>	
Composition / Purity: <u> </u>		Test Start Date: <u>8-10-93</u>
Solvent: <u>N/A</u> Conc. <u> </u>		Test End Date: <u>8-15-93</u>
		Test Units: (i.e., mg/ml) <u>mg/ml</u>

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.3	7.1	7.1	7.1	X
Control	X	7.4	7.5	7.4	7.4
Highest Conc.	X	6.9	7.2	6.8	8.0

FETAX CONTROL	MORTALITY	MALFORMATION
$\frac{\text{No. Dead or Malformed}}{\text{Total Number}} \times 100 = \%$	$\frac{1}{100} \times 100 = 1\%$	$\frac{9}{99} \times 100 = 9.1\%$
Solvent Control: <i>N/A</i>	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Control Length: <i>0.98 ± .105 mm</i>	Solvent Control Length: \quad mm	
Minimum Concentration to Inhibit Growth (MCIG): <i>.5 mg/ml</i>		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.7 mg/ml</u>	<u>0.3 mg/ml</u>	<u>Williams</u>
LOEL	<u>1.0 mg/ml</u>	<u>0.5 mg/ml</u>	<u>Williams</u>
LC ₅₀ <u>1.06 mg/ml</u>	EC ₅₀ <u>0.70 mg/ml</u>		
95% Confidence Limits <u>0.93 - 1.22 mg/ml</u>		95% Confidence Limits <u>0.51 - 0.96 mg/ml</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) = 1.51

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2.5 mg/ml	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

FETAX SUMMARY SHEET

Test No. 1

Test Material UNKNOWN P38

Investigator

TURLEY

Source OSU

Lab

U.MD-WREC

CAS No.

Lot No.

Test Start Date

1/21/93

Composition/Purity

Test End Date

1/25/93

Solvent

Conc.

Test Units (i.e., mg/ml)

mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.33	7.40	7.40	7.38	7.39
Control	7.61	7.63	7.66	7.62	7.69
Highest Conc.	7.40	7.42	7.44	7.48	7.48

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed			
Total Number		2 : 100 X 100 = 2 %	6 : 98 X 100 = 6 %
Solvent Control		- : - X 100 = - %	- : - X 100 = - %
Control Length 10.21 mm		Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 9.0 mg/ml			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀ 9.26	Trimmed Spearman-Kärber	EC ₅₀ 7.24	Trimmed Spearman-Kärber
95% Confidence limits (8.93-9.61)		95% Confidence Limits (6.66-7.86)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 1.28

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	— : — X 100 = — %	— : — X 100 = — %
2500 mg/L	— : — X 100 = — %	— : — X 100 = — %

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>UNKNOWN</u> <u>P3B</u>	Investigator <u>TURLEY</u>
Source <u>OSU</u>	Lab <u>U MD - WREC</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>1/21/93</u>
Solvent	Conc.
	Test End Date <u>1/25/93</u>
	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock	7.36	7.40	7.40	7.40	7.36
Control	7.71	7.65	7.60	7.71	7.63
Highest Conc.	7.59	7.57	7.55	7.56	7.57

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u>2</u> X 100 = %	<u>2</u> : <u>100</u> X 100 = <u>2</u> %	<u>6</u> : <u>98</u> X 100 = <u>6</u> %
Total Number		
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length <u>10.02</u> mm	Solvent Control Length <u> </u> mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>8.0</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀ <u>10.21</u> mg/ml Trimmed Spearman-Kärber	EC ₅₀ <u>7.69</u> mg/ml Trimmed Spearman-Kärber		
95% Confidence limits <u>(9.79 - 10.66)</u>	95% Confidence Limits <u>(7.10 - 8.33)</u>		
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>1.33</u>

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

FETAX SUMMARY SHEET

Test No. 3

Test Material UNKNOWN P3B		Investigator TURLEY
Source O.S.U.		Lab UMD-WREC
CAS No.	Lot No.	Test Start Date JAN 27, 1993
Composition/Purity		Test End Date JAN 31, 1993
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.42	7.40	7.41	7.44	7.46
Control	7.69	7.71	7.73	7.61	7.67
Highest Conc.	7.58	7.59	7.63	7.58	7.57

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	3 : 100 X 100 = 3 %	7 : 97 X 100 = 7 %
Solvent Control	: X 100 = %	: X 100 = %
Control Length 10.16 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 8.0 mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀ 10.25 mg/ml Trimmed Spearman-Kärber	EC ₅₀ 7.35 mg/ml Trimmed Spearman-Kärber		
95% Confidence limits 9.65-10.90	95% Confidence Limits 6.69-8.07		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.39

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

FETAX SUMMARY SHEET

Test No. 1

Test Material <u>P3B</u>	Investigator <u>FORT</u>
Source <u>ILS, Phase 3</u>	Lab <u>SBL</u>
CAS No. _____ Lot No. _____	Test Start Date <u>12/14/92</u>
Composition/Purity _____	Test End Date <u>12/18/92</u>
Solvent _____ Conc. _____	Test Units (i.e., ng/ml) _____

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	3.5/7.0	3.5/7.0	3.5/7.0	3.5/7.0	
Control	8.0	7.9	7.9	7.9	
Highest Conc.	7.3	7.4	7.5	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed _____		
Total Number _____ X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Solvent Control	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Control Length <u>95.0</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>15.0</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>5.0</u>	<u>5.0</u>	<u>Williams</u>
LOEL	<u>10.0</u>	<u>10.0</u>	<u>Williams</u>
LC ₅₀ <u>12.20</u>	EC ₅₀ <u>11.94</u>		
95% Confidence Limits <u>11.33 - 13.13</u>		95% Confidence Limits <u>10.59 - 13.46</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.07

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX SUMMARY SHEET

(DE)

Test Material P3-B		Test No. 2
Source FETAX ILS		Investigator Fort
CAS No.	Lot No.	Lab SBL
Composition/Purity		Test Start Date 2/28/93
Solvent	Conc.	Test End Date 3/4/93
		Test Units (i.e., mg/ml) mg/ml

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control	7.8	7.8	7.7	7.8	
Highest Conc.	5.0/7.0	5.2/7.0	4.9/7.0	5.1/7.0	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
Solvent Control	0 : 100 X 100 = 0 %	1 : 100 X 100 = 1 %
Control Length 94.3 mm		
Minimum Concentration to Inhibit Growth (MCIG) 16.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	11.0	11.0	Williams
LOEL	12.0	12.0	"
LC ₅₀	14.26	EC ₅₀	13.47
95% Confidence limits 14.00 - 14.54		95% Confidence Limits 13.14 - 13.80	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.06

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material P3-B

Investigator Fort

Source FETAX ILS

Lab SBL

CAS No.

Lot No.

Test Start Date 3/2/93

Composition/Purity

Test End Date 3/6/93

Solvent

Conc.

Test Units (i.e., mg/ml) mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock					
Control	8.0	7.9	7.9	7.9	
Highest Conc.	5.0/7.0	4.9/7.0	4.8/7.0	5.1/7.0	

Highest Conc.		5.0 / 1.0		1.0 / 1.0	
FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed					
Total Number		X 100 = %		X 100 = %	
		: X 100 = %		: X 100 = %	
Solvent Control		: X 100 = %		: X 100 = %	
Control Length 90.0 mm		Solvent Control Length mm			
Minimum Concentration to Inhibit Growth (MCIG)		13.0			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	12.0	12.0	Williams
LOEL	13.0	13.0	"
LC ₅₀	14.36	EC ₅₀	13.68
95% Confidence limits 14.08 - 14.65		95% Confidence Limits 13.43 - 13.92	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

1.05

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

β -AMINOPROPIONITRILE

ORIGINAL DATA TABLE WITH MATERIALS

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Table 3. Results of the Interlaboratory Study for β -aminopropionitrile.

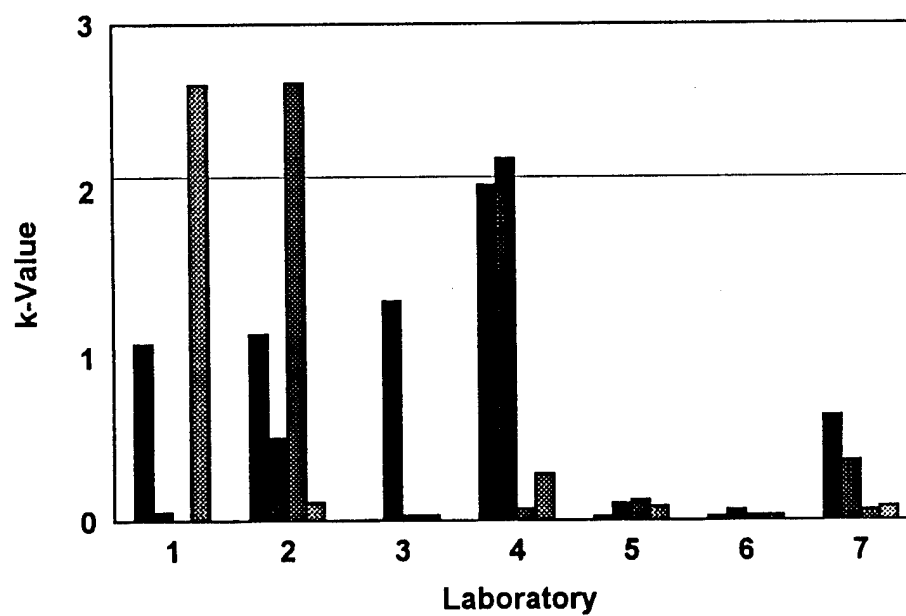
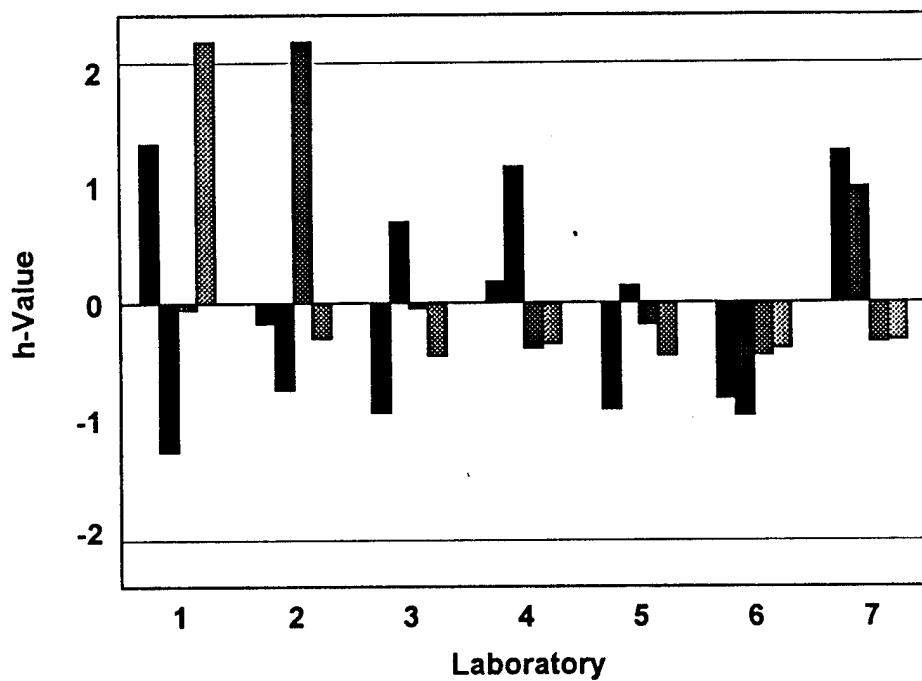
Laboratory	Replicate (n)	Mean		LC50 CV ¹ (%)	EC50 (mg/L)	Mean		EC50 CV (%)	Mean TI ²	TI CV (%)	Mean		MCIG ³ (mg/L)	Mean MCIG (mg/L)	MCIG CV (%)
		LC50 (mg/L)	LC50 (mg/L)			EC50 (mg/L)	EC50 (mg/L)								
1	1	93.70			0.07		0.07	25.5	1221.0	58.7	0.08		0.08		14.1
	2	45.40	79.37	30.4	0.08		0.08				0.08		0.08		
	3	99.00			0.04						0.10				
2	1	9.50			0.021		0.30	57.9	97.9	30.9	0.20		11.73		86.9
	2	65.40	29.43	86.6	0.55						25.00				
	3	13.40			0.15						10.00				
3	1	5.30			0.28		0.93	49.2	4.5	167.2	0.25		0.33		35.4
	2	2.90	4.17	23.6	1.28						0.25				
	3	4.30			1.24						0.50				
4	1	12.60			2.22		1.15	66.2	35.4	213.6	0.50		0.45		59.5
	2	105.00	40.53	112.8	0.63						0.75				
	3	4.00			0.59						0.10				
5	1	5.50			0.67		0.69	5.2	7.1	12.2	1.00		0.00133		35.4
	2	4.70	4.90	8.8	0.66						2.00				
	3	4.50			0.74						1.00				
6	1	7.70			0.21		0.20	11.8	40.1	20.6	0.10		0.20		54.0
	2	7.40	7.93	7.0	0.22						0.15				
	3	8.70			0.17						0.35				
7	1	60.00			1.10		1.07	11.7	72.2	30.8	1.00		0.67		35.4
	2	76.00	77.00	18.6	1.20						0.50				
	3	95.00			0.90						0.50				

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of β -aminopropionitrile



FETAX Summary Sheet

Test Material		P3C	Investigator	JAMES RAYBURN
Source		OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.		Test Start Date:	11-30-92
Composition/Purity			Test End Date	12-4-92
Solvent	Conc.		Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	6.5	6.6	6.6	6.5	
Control		7.6	7.2	7.2	7.3
Highest Concentration		6.6	6.7	7.3	7.4

No. Dead or Malformed			
----- X 100 = %			
Total Number		Mortality Record	Malformation Record
FETAX Control	9 : 100	X 100 = 9%	5 : 91 X 100 = 5.5%
Solvent Control		X 100 =	: X 100 =
Control Length	C20	Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)		J21 0.00008	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.075	2.5E-05	T-test
LOEL	0.1	5E-05	T-test
LC50	0.094	EC50	6.93E-05
95% CL	0.065 -- 0.135	95% Confidence limits	4.59E-05 --- 1.0E-04
Test Teratogenic Index (TI = LC50/EC50):		1351.44	
95% Confidence limits		780.52 -- 2339.95	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 2

Test Material	P3C	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-7-92
Composition/Purity		Test End Date	12-11-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.6	6.5	6.7	6.5	
Control		7.3	7.2	7.5	7.3
Highest Concentration		7.2	7.4	7.1	6.7

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	5 : 100 X 100 = 5%	5 : 95 X 100 = 5.3%
Solvent Control	: X 100 =	X 100 =
Control Length	C20	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	J2t 0.00008	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.00075	2.5E-05	T-test
LOEL	0.05	5E-05	T-test
LC50	0.045	EC50	8.26E-05
95% CL	0.033 -- 0.063	95% Confidence limits	6E-05 ---- 1.1E-04
Test Teratogenic Index (TI = LC50/EC50):		550.09	
95% Confidence limits		354.20 --	854.33

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 3

Test Material	P3C	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	12-13-92
Composition/Purity		Test End Date	12-17-92
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.7	6.7	6.6	6.6	
Control		7.7	7.4	7.2	7.4
Highest Concentration		7.3	7.4	7.3	7

No. Dead or Malformed				
_____ X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	7 : 100	X 100 = 7%	4 : 93	X 100 = 4.3%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	C20	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)		0.00010		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.075	N.A.	T-test
LOEL	0.1	1E-05	T-test
LC50	0.099	EC50	4.34E-05
95% CL	0.081 -- 0.121	95% Confidence limits	3.24E-05 --- 5.8E-05

Test Teratogenic Index (TI = LC50/EC50): 2281.47

95% Confidence limits 1602.02 -- 3249.09

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX SUMMARY SHEET

Test Material: <u>Unknown PBC</u>		Test No. <u>3-Def1</u> <i>MB</i>
Source:		Investigator: <u>Hull</u>
CAS No.	Lot No.	Laboratory: <u>Banke / OSI</u> <i>MB</i>
Composition / Purity:		Test Start Date: <u>12-17-92</u>
Solvent: Conc.		Test End Date: <u>12-21-92</u>
		Test Units: (i.e., mg/ml) <u>mg/ml</u>

	Day 1	Day 2	Day 3	Day 4	Day 5
--pH--					
Stock	7.6, 7.5	7.5, 7.7	8.1, 7.7	7.5, 7.6	X
Control	X	7.5	7.8	8.0	8.0
Highest Conc.	X	7.6	8.3	8.3	8.1

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	<u>10 : 100</u> X 100 = <u>10</u> %	<u>7 : 90</u> X 100 = <u>7.7</u> %
Solvent Control:	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length: mm	Solvent Control Length: mm	
Minimum Concentration to Inhibit Growth (MCIG): <u>0.0002</u>		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.001	0.000075	Williams
LOEL	0.01	0.0001	Williams
LC ₅₀ 0.00948		EC ₅₀ 0.000206	
95% Confidence Limits 0.00167 - 0.0535		95% Confidence Limits 0.00017 - 0.00024	
TEST TERATOGENIC INDEX (TI = LC ₅₀ /EC ₅₀) = 47.77 16.02 <i>A</i>			

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2.5 mg/ml	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX Summary Sheet

Test No. 2

Test Material	Unknown P3C	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU
CAS No.	B5	Lot No.	E5
Test Start Date:	Feb 1 1993	Test End Date	Feb 5 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.6, 7.6	7.7, 7.4	7.6, 7.2	7.6, 7.4	X
Control	X	7.4	7.7	7.7	7.6
Highest Concentration	X	7.2	7.7	7.8	7.5

No. Dead or Malformed
X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number	Mortality Record	Malformation Record
FETAX Control	8 : 100 X 100 = 8%	9 : 92 X 100 = 9.8%
Solvent Control	C19 : E19 X 100 = G19	H19 : J19 X 100 = L19
Control Length	0.9	Solvent Control Length J20
Minimum Concentration to Inhibit Growth (MCIG)	0.025	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.025	0.0003	T-test
LOEL	0.1	0.0005	T-test
LC50	6.54E-02	EC50	5.45E-04
95% CL	1.97E-02 -- 2E-01	95% Confidence limits	0.0003, 4.0E-04 ---- 0.001
Test Teratogenic Index (TI = LC50/EC50):		1.20E+02	
95% Confidence limits		3.31E+01 --	4.4E+02

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 3

Test Material	Unknown P3C	Investigator	Mendi A. Hull
Source	B4	Laboratory	Bantle / OSU <i>QMB</i>
CAS No.	B5	Lot No.	E5
Test Start Date:	Mar 8 1993	Test End Date	Mar 12 1993
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/ml
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.6	7.1	7.4	7.6	X
Control	X	7.1	7.1	6.8	6.6
Highest Concentration	X	7.5	7.4	7.0	7.0

No. Dead or Malformed		
_____ X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	3 : 100 X 100 = 3%	4 : 97 X 100 = 4.1%
Solvent Control	C19 : E19 X 100 = G19	H19 : J19 X 100 = L19
Control Length	320 1 cm	Solvent Control Length J20
Minimum Concentration to Inhibit Growth (MCIG)	0.01 0.01 mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.01	7.5E-05	T-test
LOEL	0.025	0.0001	T-test
LC50	0.013	EC50 1.51E-04	
95% CL	0.010 -- 0.018	95% Confidence limits 1E-04 --- 1.8E-04	

Test Teratogenic Index (TI = LC50/EC50):	88.57
95% Confidence limits	64.09 -- 122.40

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

			Test No.	1-P3C
Test Material	P3C	Investigator	FINCH	
Source	OSU	Laboratory	USABRDL	
CAS No.	E5	Lot No.	E5	Test Start Date: 28 JUN 93
Composition/Purity	CS			Test End Date 2 JULY 93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML	

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	E11	F11	G11	J11	
	Control		F12	G12	J11	K12
	Highest Concentration		F13	B13	J11	K13

No. Dead or Malformed					
		X 100 = %			
Total Number		Mortality Record		Malformation Record	
FETAX Control		0 : 100	X 100 = 0%	4 : 100	X 100 = 4.0%
Solvent Control		C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length		C20	Solvent Control length	J20	
Minimum Concentration to Inhibit Growth (MCIG)		J21			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used		
NOEL	0.0025	0.0001	T-test		
LOEL	0.005	0.00025	T-test		
LC50	0.00530	EC50	0.00028		
95% CL	0.00453 --	0.00620	95% Confidence limits	0.00023 --	0.00035
Test Teratogenic Index (TI = LC50/EC50):		18.65			
95% Confidence limits		14.29 --	24.34		

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality		Malformation	
5.5 mg/L	C33 : E33	X 100 = G33	H33 : J33	X 100 = L33
2500 mg/L	C34 : E34	X 100 = G34	H33 : J34	X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 2-P3C

Test Material	P3C	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	E5	Lot No.	E5
Composition/Purity	06	Test Start Date:	
Solvent	Comp.	Test End Date	
		Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	E11	F11	G11	J11	
Control		F12	G12	J11	K12
Highest Concentration		F13	B13	J11	K13

No. Dead or Malformed	
X 100 = %	
Total Number	
	Mortality Record
FETAX Control	4 : 100 X 100 = 4%
Solvent Control	C19 : E19 X 100 = G19
Control Length	C20
Minimum Concentration to Inhibit Growth (MCIG)	J21
	Malformation Record
	6 : 96 X 100 = 6.3%
	H19 : J19 X 100 = L19
	Solvent Control length J20

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.0025	0.0005	T-test
LOEL	0.005	0.0025	T-test
LC50	0.00288	EC50 0.00128	
95% CL	0.00237 -- 0.00350	95% Confidence limits 0.00106 -- 0.00154	

Test Teratogenic Index (TI = LC50/EC50):	2.25
95% Confidence limits	1.72 -- 2.94

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test Material		P3C	Investigator	FINCH
Source		OSU	Laboratory	USABRDL
CAS No.	B5	Lot No.	E5	Test Start Date:
Composition/Purity		C8	Test End Date	
Solvent		Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	E11	F11	G11	J11	
Control		F12	G12	J11	K12
Highest Concentration		F13	B13	J11	K13

No. Dead or Malformed				
_____ X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	3 : 100	X 100 = 3%	0 : 97	X 100 = 0.0%
Solvent Control	C19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	C20	Solvent Control length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	J21			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.0025	0.0005	T-test
LOEL	0.005	0.00075	T-test
LC50	0.00427	EC50	0.00124
95% CL	0.00378 -- 0.00482	95% Confidence limits	0.00103 -- 0.00149
Test Teratogenic Index (TI = LC50/EC50):			3.45
95% Confidence limits			2.76 -- 4.30

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality		Malformation	
5.8 mg/L	C33 : E33	X 100 = G33	H33 : J33	X 100 = L33
2500 mg/L	C34 : E34	X 100 = G34	H33 : J34	X 100 = L34

CL= Confidence limits

FETAX SUMMARY SHEET

Test Material: P3C-1		Test No. Definitive #1
Source: Bantle		Investigator: Gillett
CAS No.	Lot No.	Laboratory: CERL
Composition / Purity:		Test Start Date: 09/27/93
Solvent: Conc.		Test End Date: 10/01/93
		Test Units: (i.e., mg/ml) 1 mg/ml

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	7.0	7.1	7.0	
Control	X	7.5	7.6	7.3	
Highest Conc.	X	7.6	7.5	not taken	

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	____:____ X 100 = 46%	____:____ X 100 = 82%
Solvent Control:	____:____ X 100 = ____%	____:____ X 100 = ____%
Control Length: 9.5645 mm	Solvent Control Length: ____ mm	
Minimum Concentration to Inhibit Growth (MCIG): 0.0005 mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL		0.0006	Williams
LOEL		0.0008	Williams
LC ₅₀ .0126		EC ₅₀ .00222	
95% Confidence Limits .0111 - .0144		95% Confidence Limits .00200 - .00247	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) - 5:6757

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%
2.5 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%

FETAX SUMMARY SHEET

Test Material: <u>P3C-2</u>		Test No. _____
Source: <u>Bantle</u>		Investigator: <u>G. Heff</u>
CAS No. _____	Lot No. _____	Laboratory: <u>CERL</u>
Composition / Purity: _____		Test Start Date: <u>10/11/93</u>
Solvent: _____		Test End Date: <u>10/15/93</u>
Conc. _____		Test Units: (i.e., mg/ml)

	Day 1	Day 2	Day 3	Day 4	Day 5
Stock <u>--pH--</u> <u>(DCU)</u> <u>7.04</u>	7.0	7.0	7.0	7.1	X
Control	X	7.6	7.4	7.2	7.5
Highest Conc.	X	7.5	7.7	7.7	7.6

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	____:____ X 100 = <u>3</u> %	____:____ X 100 = <u>4</u> %
Solvent Control:	____:____ X 100 = ____ %	____:____ X 100 = ____ %
Control Length: <u>0.9448</u> mm	Solvent Control Length: _____ mm	
Minimum Concentration to Inhibit Growth (MCIG): <u>0.00075</u> mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>.025</u>	<u>.005</u> Kruskal-Wallis	<u>Williams</u>
LOEL	<u>.05</u>	<u>.01</u> Kruskal-Wallis	<u>Williams</u>
LC ₅₀ <u>.105</u>	EC ₅₀ <u>.000631</u>		
95% Confidence Limits <u>.090 - .123</u>		95% Confidence Limits <u>.000412 - .000964</u>	

TEST TERATOGENIC INDEX (TI = LC_{50}/EC_{50}) = $\frac{.105}{.000631}$ = 166.4 - .000412 - .000964

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	____:____ X 100 = ____ %	____:____ X 100 = ____ %
2.5 mg/ml	____:____ X 100 = ____ %	____:____ X 100 = ____ %

FETAX SUMMARY SHEET

Test Material: <u>p3c</u>		Test No. <u>Ref. #3</u>
Source: <u>Battle</u>		Investigator: <u>Gillott/wilborn</u>
CAS No.	Lot No.	Laboratory: <u>ERL-C</u>
Composition / Purity:		Test Start Date: <u>10/22/93</u>
Solvent:	Conc.	Test End Date: <u>10/26/93</u>
		Test Units:(i.e., mg/ml)

	Day 1	Day 2	Day 3	Day 4	Day 5
--pH--					
Stock	7.2	7.1	7.1	7.1	7.6
Control	—	Not taken	7.2	7.2	7.6
Highest Conc.	—	Not taken	7.8	7.7	7.6

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	___:___ X 100 = ___%	___:___ X 100 = ___%
Solvent Control:	___:___ X 100 = ___%	___:___ X 100 = ___%
Control Length: <u>10.0359</u> mm	Solvent Control Length: _____ mm	
Minimum Concentration to Inhibit Growth (MCIG): <u>0.0001</u> mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	.001	.0001	Williams
LOEL	.005	.0005	Williams
LC ₅₀ .004		EC ₅₀ .000589	
95% Confidence Limits .00358 - .00638		95% Confidence Limits .000467 - .000744	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) = 6.79

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	___:___ X 100 = ___%	___:___ X 100 = ___%
2.5 mg/ml	___:___ X 100 = ___%	___:___ X 100 = ___%

FETAX SUMMARY SHEET

Test No. 1

Test Material <u>Unknown P3C</u>		Investigator <u>TURLEY</u>
Source <u>OSU</u>		Lab <u>U.M.D.-WREC</u>
CAS No.	Lot No.	Test Start Date <u>2/2/93</u>
Composition/Purity		Test End Date <u>2/6/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	6.99	6.92	6.87	6.88	6.91
Control	7.67	7.69	7.74	7.70	7.67
Highest Conc.	7.56	7.51	7.49	7.52	7.55

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>4</u> : <u>100</u> X 100 = <u>4</u> %	<u>5</u> : <u>100</u> X 100 = <u>5</u> %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length <u>10.01</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.0</u> mg/L		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	4.0	0.50	Benferroni T-test
LOEL	5.0	0.60	Benferroni T-test
LC ₅₀ <u>0.549</u> mg/L Trimmed Spearman-Kärber	EC ₅₀ <u>0.67</u> mg/L Trimmed Spearman-Kärber		
95% Confidence limits <u>5.04 - 5.98</u>	95% Confidence Limits <u>0.59 - 0.75</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 8.19

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>Unknown P3C</u>		Investigator <u>TURLEY</u>
Source <u>O.S.U</u>		Lab <u>U.MD-WREC</u>
CAS No.	Lot No.	Test Start Date <u>2/9/93</u>
Composition/Purity		Test End Date <u>2/13/93</u>
Solvent <u>None</u>	Conc.	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	6.99	6.90	6.89	6.94	6.98
Control	7.71	7.70	7.68	7.72	7.68
Highest Conc.	7.60	7.58	7.56	7.48	7.56

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>2</u> : <u>100</u> X 100 = <u>2</u> %	<u>7</u> : <u>100</u> X 100 = <u>7</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length <u>10.07 mm</u>	Solvent Control Length <u>mm</u>	
Minimum Concentration to Inhibit Growth (MCIG) <u>2.00 mg/L</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	4.00 mg/L	0.25 mg/L	Bonferroni T-test
LOEL	5.00 mg/L	0.50 mg/L	Bonferroni T-test
LC ₅₀ <u>4.70</u> (Trimmed Spearman-Kärber)	EC ₅₀ <u>0.66</u> mg/L (Trimmed Spearman-Kärber)		
95% Confidence limits <u>4.49-4.91</u>	95% Confidence Limits <u>0.57-0.77</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 7.12

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>Unknown P3C</u>	Investigator <u>TURLEY</u>
Source <u>O.S.U</u>	Lab <u>U.MD-WREC</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>2/20/93</u>
Solvent	Conc.
	Test End Date <u>2/24/93</u>
	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	6.94	6.87	6.92	6.90	6.98
Control	7.68	7.69	7.65	7.63	7.72
Highest Conc.	7.51	7.48	7.49	7.51	7.53

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>3</u> : <u>100</u> X 100 = <u>3</u> %	<u>7</u> : <u>100</u> X 100 = <u>7</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length <u>10.09</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.00</u> mg/L		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL Homogeneity	FAIL Homogeneity	Bonferroni T-test
LOEL	FAIL Homogeneity	FAIL Homogeneity	Bonferroni T-test
LC ₅₀ <u>4.46</u> mg/L Trimmed Spearman-Kärber	EC ₅₀ <u>0.74</u> mg/L Trimmed Spearman-Kärber		
95% Confidence limits <u>4.08 - 4.89</u>	95% Confidence Limits <u>0.63 - 0.88</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 6.03

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test Material <u>P3C</u>		Investigator <u>D.A. DANSEN</u>
Source		Lab <u>UT/CVM</u>
CAS No.	Lot No.	Test Start Date <u>12/8/92</u>
Composition/Purity		Test End Date <u>12/12/92</u>
Solvent	Conc.	Test Units (l.o., mg/ml) <u>mg/L</u>

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.09	7.11	7.09	7.09	—
Control	7.91	7.67	7.64	7.62	7.63
Highest Conc.	—	7.80	7.84	7.84	7.83

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>3</u> : <u>100</u> X 100 = <u>3</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.0001</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>< 0.006</u> <u>btl</u>	<u>0.0001</u> <u>btl</u>	
LOEL	<u>0.006</u> <u>btl</u>	<u>0.0002</u> <u>btl</u>	
LC ₅₀	<u>0.0077</u> <u>TSK</u>	EC ₅₀ <u>0.00021</u> <u>TLW</u> <u>0.00018</u> <u>TSK</u>	
95% Confidence limits <u>(0.0073 - 0.0082)</u> <u>TSK</u>		95% Confidence Limits <u>(0.00008 - 0.00051)</u> <u>(0.00008 - 0.00051)</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		<u>42.8</u> - TSK - EC ₅₀ <u>36.7</u> - TLW - EC ₅₀	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

D.A.D.
12/14/92

FETAX SUMMARY SHEET

Test No. 2

Test Material <i>P3C</i>		Investigator <i>B. Dawson</i>
Source		Lab <i>UT/CM</i>
CAS No.	Lot No.	Test Start Date <i>11/1/53</i>
Composition/Purity		Test End Date <i>1/8/53</i>
Solvent	Conc.	Test Units (i.e., mg/ml) <i>mg/ml</i>

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.07	7.05	7.12	7.04	—
Control	7.79	7.75	7.51	7.55	7.52
Highest Conc.	—	7.86	7.76	7.61	7.61

FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed		X 100 = %			
Total Number		0 : 100 X 100 = 0 %		2 : 100 X 100 = 2 %	
Solvent Control		: X 100 = %		: X 100 = %	
Control Length mm		Solvent Control Length mm			
Minimum Concentration to Inhibit Growth (MCIG) <i>0.00015</i>					

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.04 <i>both</i>	0.0001	
LOEL	0.06	0.00015 <i>both</i>	
LC ₅₀	0.0074	EC ₅₀ 0.000218	<i>LEW</i>
95% Confidence limits (0.0070-0.0077)		95% Confidence Limits (0.00019 - 0.00025)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

33.9

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <i>not</i>	: X 100 = %	: X 100 = %
2500 mg/L <i>live</i>	: X 100 = %	: X 100 = %

D. J. D.
1/1/53

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3C	Investigator	D.A. DAWSON
Source		Lab	UT/CWH
CAS No.	Lot No.	Test Start Date	1/11/93
Composition/Purity		Test End Date	1/15/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.08	7.09	7.05	7.07	—
Control	7.82	7.72	7.61	7.59	7.51
Highest Conc.	—	7.81	7.75	7.67	7.27

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	1 : 100 X 100 = 1 %	1 : 99 X 100 = 1.01 %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 0.00035		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.006 beth	< 0.0001 WITH.	
LOEL	0.008 beth	0.0001 WITH.	
LC ₅₀	0.011 - TSK 0.0097 - EPA	EC ₅₀	0.000162 - LEW 0.00017 - TSK 0.000165 - EPA
95% Confidence Limits	(0.0091 - 0.0166) - TSK (0.0071 - 0.014) - EPA	95% Confidence Limits	(0.00014 - 0.00018) - LEW (0.00015 - 0.00018) - TSK (0.00015 - 0.00018) - EPA
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
64.7 - TSK 52.7 - EPA			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L not	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L clasp	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

D.A.D.
1/15/93

FETAX SUMMARY SHEET

Test No. 1

Test Material	P3C	Investigator	FORT
Source	ILS, Phase 3	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/14/92
Solvent		Conc.	
		Test End Date	12/18/92
		Test Units (i.e., mg/ml)	

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.5	7.5	7.4	7.5	
Control	8.0	8.0	7.9	7.9	
Highest Conc.	7.6	7.7	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
	$\frac{\text{No. Dead}}{\text{Total Number}} \times 100 = \%$	$\frac{\text{No. Malformed}}{\text{Total Number}} \times 100 = \%$
Solvent Control		
Control Length 95.0 mm	Solvent Control Length	mm
Minimum Concentration to Inhibit Growth (MCIG)	0.001	

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.005	0	Williams
LOEL	0.01	0.0005	Williams
LC ₅₀	0.06	EC ₅₀	0.0011
95% Confidence limits	0.05-0.07	95% Confidence Limits	0.0009 - 0.0015

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 54.6

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	$\frac{\text{No. Dead}}{\text{Total Number}} \times 100 = \%$	$\frac{\text{No. Malformed}}{\text{Total Number}} \times 100 = \%$
2500 mg/L	$\frac{\text{No. Dead}}{\text{Total Number}} \times 100 = \%$	$\frac{\text{No. Malformed}}{\text{Total Number}} \times 100 = \%$

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>P3C</u>		Investigator <u>Fort</u>
Source <u>FETAX ILS</u>		Lab <u>SBL</u>
CAS No.	Lot No.	Test Start Date <u>1/21/93</u>
Composition/Purity		Test End Date <u>1/25/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	<u>7.2</u>	<u>7.3</u>	<u>7.4</u>	<u>7.4</u>	
Control	<u>7.8</u>	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>	
Highest Conc.	<u>7.5</u>	<u>7.5</u>	<u>7.5</u>	<u>7.6</u>	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u> </u> X 100 = %		
Total Number	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length <u>90.3</u> mm	Solvent Control Length <u> </u> mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.0005</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.005</u>	<u>0.0005</u>	<u>Williams</u>
LOEL	<u>0.05</u>	<u>0.00075</u>	<u>Williams</u>
LC ₅₀	<u>0.076</u>	EC ₅₀ <u>0.0012</u>	
95% Confidence limits <u>0.063 - 0.092</u>		95% Confidence Limits <u>0.0010 - 0.0014</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 63.33

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

FETAX SUMMARY SHEET

Test No. 3	
Test Material P3C	Investigator Fort
Source FETAX ILS	Lab SBL
CAS No. _____ Lot No. _____	Test Start Date 1/23/93
Composition/Purity _____	Test End Date 1/27/93
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) mg/ml

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.3	7.2	7.3	7.4	
Control	7.9	8.0	7.9	7.9	
Highest Conc.	7.7	7.8	7.8	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed _____		
Total Number _____ X 100 = %	0 : 100 X 100 = 100 %	0 : 100 X 100 = 0 %
Solvent Control _____	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length 91.6 mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) 0.0005		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.005	0.0005	Williams Test
LOEL	0.05	0.00075	Williams Test
LC ₅₀	0.095	EC ₅₀	0.0009
95% Confidence Limits 0.083 - 0.107		95% Confidence Limits 0.0008 - 0.0011	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | **105.56**

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

SODIUM ACETATE

PHASE III COMPOUND D DATA (P3D) SODIUM ACETATE

FETAX ILS STUDY; 7 LABS, 8 LEVELS, 3 REPLICATES

PAGE 1

units in mg/ml

ORIGINAL DATA TABLE WITH MATERIALS

LAB	LC50	CI LO	CI HI	EC50	CI LO	CI HI	MCIG	TI	LOEC(MORT)	LOEC(MALF)	SUBTOTALS:	STD:	CV:
BANTLE/ RAYBURN	1	6.96	5.34	9.07	2.32	4.10	1.00	3.0	12.00	1.00	LC50	6.21	10.6
	2	6.30	5.29	7.49	1.41	3.05	6.00	4.5	0.25	0.20	EC50	2.37	33.8
	3	5.36	4.62	6.21	3.37	4.43	2.00	1.6	4.00	4.00	MCIG	3.00	72.0
											TI	2.62	45.2
BANTLE/ HULL	1	11.20	9.52	13.17	2.01	3.73	0.50	5.6	8.00	0.50	LC50	10.96	2.3
	2	11.07	10.16	12.06	1.18	1.63	0.50	9.4	12.00	0.25	EC50	1.45	27.1
	3	10.62	10.14	11.13	1.17	1.62	0.50	9.1	12.00	0.75	MCIG	0.50	0.0
											TI	7.54	22.9
FINCH	1	12.00	10.84	13.29	5.12	6.33	10.00	2.3	14.00	4.00	LC50	11.69	4.2
	2	11.00	9.27	13.06	7.64	8.30	9.00	1.4	12.00	9.00	EC50	7.21	21.7
	3	12.06	11.42	12.74	8.88	9.55	9.00	1.4	11.00	8.00	MCIG	9.33	5.1
											TI	1.62	26.2
LINDER/ BUCHWALTER	1	11.69	10.98	12.44	5.23	6.25	1.00	2.2	13.00	1.00	LC50	9.72	17.4
	2	9.92	9.49	10.38	6.62	7.26	1.00	1.5	9.00	5.00	EC50	6.12	10.3
	3	7.56	7.18	7.97	6.52	7.21	3.00	1.5	9.00	0.25	MCIG	1.67	56.6
											TI	1.59	20.8
BURTON/ TURLEY	1	7.76	7.49	8.04	7.92	8.61	8.00	1.0	7.00	7.00	LC50	7.05	13.3
	2	7.67	7.23	8.13	8.26	8.71	8.00	0.9	6.00	7.00	EC50	7.50	11.3
	3	5.72	5.42	6.02	6.31	6.83	7.00	0.9	4.00	6.00	MCIG	7.67	6.1
											TI	0.94	5.0
DAWSON	1	12.32	11.90	12.80	8.77	9.40	5.00	1.4	7.00	7.00	LC50	11.99	2.2
	2	11.68	10.30	13.20	9.23	12.10	5.00	1.3	10.00	5.00	EC50	8.74	4.8
	3	11.96	11.70	12.30	8.21	8.60	7.00	1.5	10.00	7.00	MCIG	5.67	16.6
											TI	1.37	6.0
STOVER/ FORT	1	8.91	7.68	10.35	1.55	1.99	0.50	5.8	5.00	0.05	LC50	7.23	19.5
	2	7.31	6.39	8.36	2.30	2.69	7.50	3.2	2.50	1.00	EC50	1.64	30.6
	3	5.46	4.63	6.43	1.08	1.19	2.50	5.1	2.50	1.00	MCIG	3.50	84.1
											TI	4.40	25.0

GRAND TOTAL

LAB	LC50	CI LO	CI HI	EC50	CI LO	CI HI	MCIG	TI	LOEC (mort)	LOEC (mal)	STD:	CV:
											2.43	26.2
											2.9744	59.4
											3.33	74.4
											2.51	135.8
											3.85	47.5
											3.05835	86.7

Table 4. Results of the Interlaboratory Study for Sodium Acetate.

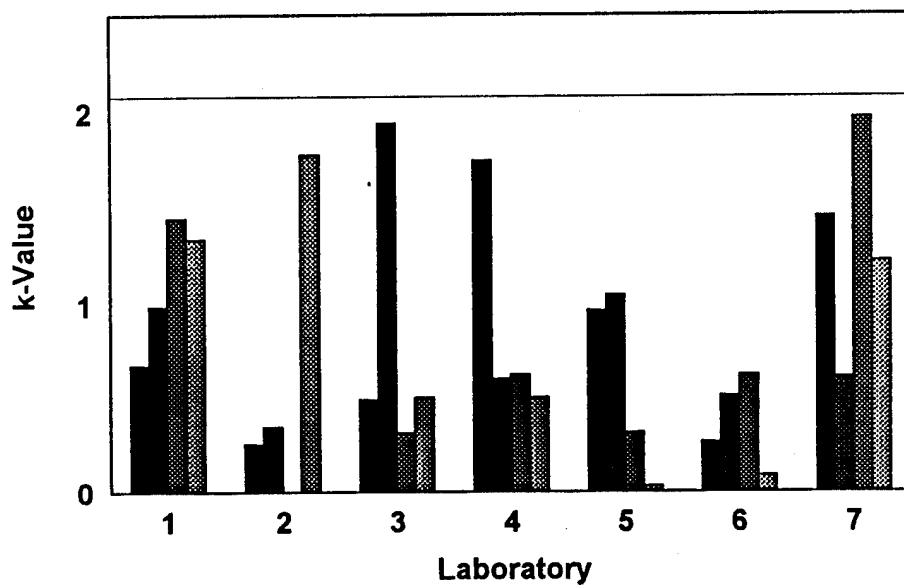
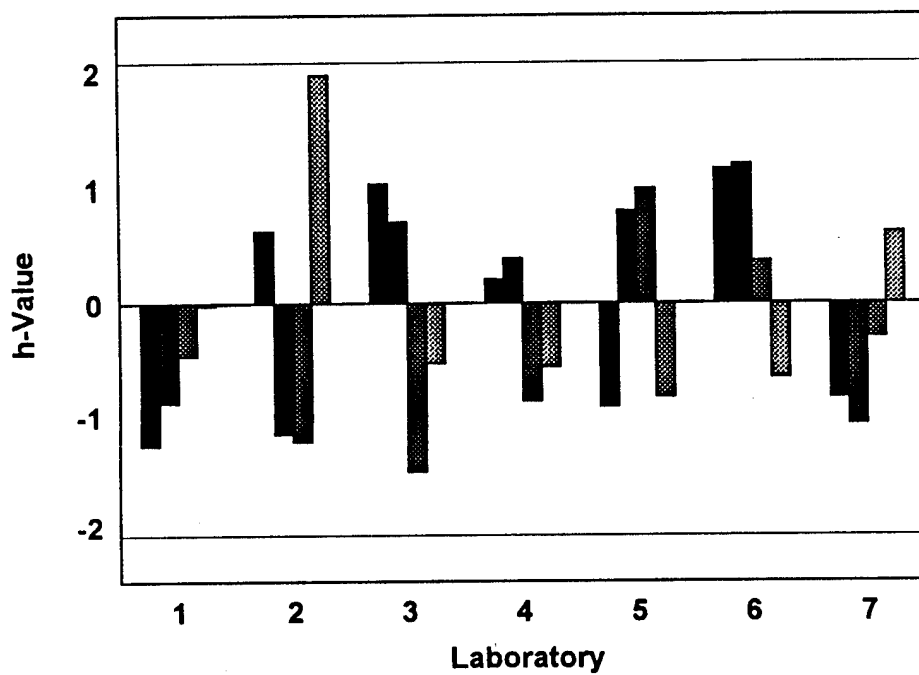
Laboratory	Replicate (n)	Mean LC50		Mean EC50		Mean TI		Mean MCIG ³		Mean MCIG	
		LC50 (mg/ml)	CV ¹ (%)	EC50 (mg/ml)	CV (%)	TI ² (%)	CV (%)	MCIG ³ (mg/ml)	CV (%)	MCIG (mg/ml)	CV (%)
1	1	6.96		2.32				1.0			
	2	6.30	10.6	1.41	33.8	2.6	44.8	6.0		3.00	72.0
	3	5.36		3.37				2.0			
2	1	11.20		2.01				0.5			
	2	11.07	2.3	1.18	27.1	7.5	22.9	0.5		0.50	0.0
	3	10.62		1.17				0.5			
3	1	12.00		5.12				10.0			
	2	11.00	4.2	7.64	21.7	1.6	27.6	9.0		9.33	5.1
	3	12.06		8.88				9.0			
4	1	11.69		5.23				1.0			
	2	9.92	17.4	6.62	10.3	1.6	28.3	1.0		1.67	56.6
	3	7.56		6.52				3.0			
5	1	7.76		7.92				8.0			
	2	7.67	13.3	8.26	11.3	0.9	3.3	8.0		7.67	6.1
	3	5.72		6.31				7.0			
6	1	12.32		8.77				5.0			
	2	11.68	2.2	9.23	4.8	1.4	5.9	5.0		5.67	16.6
	3	11.96		8.21				7.0			
7	1	8.91		1.55				0.50			
	2	7.31	19.5	2.30	30.6	4.4	24.7	7.50		3.50	84.1
	3	5.46		1.08				2.50			

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of Sodium Acetate



FETAX Summary Sheet

Test No. DEF 1

Test Material	P3D	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	3-25-93
Composition/Purity		Test End Date	3-29-93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.9	7.9	7.8	7.8	
Control		7.8	7.5	7.3	7.1
Highest Concentration		7.6	7.3	7.2	7.1

No. Dead or Malformed
_____ X 100 = %
Total Number
FETAX Control
Solvent Control

Mortality Record		Malformation Record	
9 : 100	X 100 = 9%	6 : 91	X 100 = 6.6%
:	X 100 =	:	X 100 =

Control Length	220 9.18	Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)		327	1 mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	0.75	T-test
LOEL	12	1	T-test
LC50	6.961	EC50	2.323
95% CL	5.340 -- 9.074	95% Confidence limits	1.316 ---- 4.101

Test Teratogenic Index (TI = LC50/EC50):	3.00
95% Confidence limits	1.60 -- 5.61

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 2

Test Material	P3D	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	3-29-93
Composition/Purity		Test End Date	4-2-93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.8	7.8	7.7	7.7	
Control		7.7	7.5	7.4	7.2
Highest Concentration		7.7	7.5	7.3	7.1

No. Dead or Malformed	
_____ X 100 = %	
Total Number	
FETAX Control	
Solvent Control	
Control Length	9.688 mg/ml
Minimum Concentration to Inhibit Growth (MCIG)	6 mg/ml

MALFORMATION EXCEED ASTM LIMIT

Mortality Record	Malformation Record
10 : 100 X 100 = 10%	7 : 90 X 100 = 7.8%
: X 100 =	: X 100 =

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	1	T-test
LOEL	0.25	2	T-test
LC50	6.296	EC50	1.409
95% CL	5.293 -- 7.489	95% Confidence limits	0.649 --- 3.059

Test Teratogenic Index (TI = LC50/EC50):	4.47
95% Confidence limits	2.02 -- 9.89

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. DEF 3

Test Material	P3D	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	4-19-93
Composition/Purity		Test End Date	4-23-93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.8	7.8	7.7	7.7	
Control		7.7	7.5	7.4	7.2
Highest Concentration		7.7	7.5	7.3	7.1

No. Dead or Malformed		
_____ X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	8 : 100 X 100 = 8%	9 : 92 X 100 = 9.8%
Solvent Control	: X 100 =	: X 100 =
Control Length 9.23	Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	2 mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	2	2	T-test
LOEL	4	4	T-test
LC50	5.356	EC50	3.374
95% CL	4.617 -- 6.213	95% Confidence limits	2.570 ---- 4.429
Test Teratogenic Index (TI = LC50/EC50):		1.59	
95% Confidence limits		1.16 -- 2.16	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 1

Test Material	P3D	Investigator	Mendi A. Hull
Source	Sigma	Laboratory	OSU Bantle
CAS No.	Lot No.	Test Start Date:	8 Mar 93
Composition/Purity		Test End Date	12 Mar 93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	8.1	8	8.2	8	
Control		7.6	7.4	7	7.1
Highest Concentration		7.8	7.5	7	8

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	3 : 100	X 100 = 3%	7 : 97	X 100 = 7.2%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	1 cm	Solvent Control Length (mm)		
Minimum Concentration to Inhibit Growth (MCI)	0.5	mg/ml		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used	
NOEL	6	0.25	T-test	
LOEL	8	0.5	T-test	
LC50	11.197	EC50	2.006	
95% CL	9.517 -- 13.173	95% Confidence limits	1.078 ---- 3.730	
Test Teratogenic Index (TI = LC50/EC50):		5.58		
95% Confidence limits		2.94 --	10.60	

FETAX Summary Sheet

Test No. 2

Test Material	P3D	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU
CAS No.	Lot No.	Test Start Date:	Mar 15 1993
Composition/Purity		Test End Date	Mar 19 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	8.1	8.0	8.2	8.0	X
Control	X	7.6	7.4	7.0	7.1
Highest Concentration	X	7.8	7.5	7.0	8.0

No. Dead or Malformed	
_____ X 100 = %	
Total Number	
FETAX Control	
Solvent Control	
Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	

Mortality Record		Malformation Record	
10 : 100	X 100 = 10%	6 : 90	X 100 = 6.7%
:	X 100 =	:	X 100 =
Solvent Control Length			
0.5 mg/ml			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	N.A.	T-test
LOEL	12	0.25	T-test
LC50	11.071	EC50	1.176
95% CL	10.161 -- 12.063	95% Confidence limits	0.849 ---- 1.630
Test Teratogenic Index (TI = LC50/EC50):		9.41	
95% Confidence limits		6.72 --	13.19

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 3

Test Material	P3D	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU
CAS No.	Lot No.	Test Start Date:	Jun 7 1993
Composition/Purity		Test End Date	Jun 11 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	8.1	8.0	8.2	8.0	X
Control	X	7.6	7.4	7.0	7.1
Highest Concentration	X	7.8	7.5	7.0	8.0

No. Dead or Malformed		
X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control	2 : 100 X 100 = 2%	7 : 98 X 100 = 7.1%
Control Length 1 cm	Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	0.5 mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	0.5	T-test
LOEL	12	0.75	T-test
LC50	10.623	EC50	1.169
95% CL	10.139 -- 11.130	95% Confidence limits	0.842 --- 1.623
Test Teratogenic Index (TI = LC50/EC50):		9.09	
95% Confidence limits		6.53 -- 12.66	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	: X 100 =	: X 100 =
2500 mg/L	: X 100 =	: X 100 =

CL= Confidence limits

FETAX Summary Sheet

Test No. 1(200-01)

Test Material	P3D	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	Lot No.	Test Start Date:	21 JUN 93
Composition/Purity		Test End Date	25 JUN 93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	8.6	8.6	8.6	8.6	
Control		7.7	7.7	7.8	7.7
Highest Concentration		8.2	8.1	8.2	8.1

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	1 : 100 X 100 = 1%	5 : 99 X 100 = 5.1%
Solvent Control	X 100 = -	X 100 = -
Control Length	9.567	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	10.0	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	12	2	T-test
LOEL	14	4	T-test
LC50	12.000 (?)	EC50	5.116 (?)
95% CL	10.835 -- 13.290	95% Confidence limits	4.139 --- 6.325
Test Teratogenic Index (TI = LC50/EC50):		2.35	
95% Confidence limits		1.85 -- 2.97	

CL = Confidence limits

FETAX Summary Sheet

Test No. 2(200-02)

Test Material	P3D	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	28 JUN 93
Solvent	-	Test End Date	2 JULY 93
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	8.6	8.6	8.7	8.7	
Control		8.0	7.9	7.7	7.8
Highest Concentration		8.2	8.2	8.4	8.3

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	1 : 100 X 100 = <input checked="" type="checkbox"/> 1%	5 : 99 X 100 = 5.1% <input checked="" type="checkbox"/>
Solvent Control	- : - X 100 = -	- : - X 100 = -
Control Length	9.361	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	9.0 <input checked="" type="checkbox"/>	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	11	6	T-test
LOEL	12	9	T-test
LC50	11.000 <input checked="" type="checkbox"/>	EC50	7.640 <input checked="" type="checkbox"/> ~8.3 <input checked="" type="checkbox"/> (?)
95% CL	9.269 - 13.055	95% Confidence limits	7.033 --- 8.300
Test Teratogenic Index (TI = LC50/EC50): ~1.3 (?) 1.44			
95% Confidence limits 1.19 -- 1.74			

CL = Confidence limits

FETAX Summary Sheet

Test No. 3(200-03)

Test Material	P3D	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B5	Lot No.	E5
Test Start Date	28 JUN 93		
Composition/Purity	CB	Test End Date	2 JULY 93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	E11	F11	G11	J11	
Control		F12	G12	J11	K12
Highest Concentration		F13	B13	J11	K13

No. Dead or Malformed				
_____ X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	1 : 100	X 100 = 1%	2 : 99	X 100 = 2.0%
Solvent Control	G19 : E19	X 100 = G19	H19 : J19	X 100 = L19
Control Length	G20	Solvent Control Length	J20	
Minimum Concentration to Inhibit Growth (MCIG)	J21 9.0			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	5	T-test
LOEL	11	8	T-test
LC50	12.061	EC50	8.882
95% CL	11.418 -- 12.740	95% Confidence limits	8.260 ---- 9.551

Test Teratogenic Index (TI = LC50/EC50):	1.36
95% Confidence limits	1.24 -- 1.49

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

C58 CT8
C59 CT9

FETAX SUMMARY SHEET

not signed

Test Material: P3D		Test No. Definitive #1
Source: Bottle		Investigator: Gillett
CAS No.	Lot No.	Laboratory: CERL
Composition / Purity:		Test Start Date: 11/01/93
Solvent: Conc.		Test End Date: 11/05/93
		Test Units: (i.e., mg/ml) <i>mg/ml</i>

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	7.0	7.0	7.1	X
Control	X	7.5	7.7	8.0	7.8
Highest Conc.	X	7.6	7.6	7.8	7.7

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	<i>No Control</i> ____:____ X 100 = ____%	____:____ X 100 = ____%
Solvent Control:	____:____ X 100 = ____%	____:____ X 100 = ____%
Control Length: 9.0739 mm	Solvent Control Length: ____ mm	
Minimum Concentration to Inhibit Growth (MCIG): 1.0 mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	11.0	0.5	Williams
LOEL	13.0	1.0	Williams
LC ₅₀	11.69	EC ₅₀	5.23
95% Confidence Limits	10.98-12.44	95% Confidence Limits	4.37-6.25

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) - 2.23

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%
2.5 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%

FETAX SUMMARY SHEET

Test Material: P3D		Test No. Def. #4
Source: Bantle		Investigator: Gillett
CAS No.	Lot No.	Laboratory: CERL
Composition / Purity:		Test Start Date: 12/09/93
Solvent: Conc.		Test End Date: 12/13/93
		Test Units: (i.e., mg/ml) mg/ml

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	7.2	7.1	7.1	—
Control	7.3 ^{7.4}	7.4	7.3	7.5	7.5
Highest Conc.	7.3 ^{7.4}	7.5	7.4	7.7	7.4

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	____:____ X 100 = ____%	____:____ X 100 = ____%
Solvent Control:	____:____ X 100 = ____%	____:____ X 100 = ____%
Control Length: 9.7844 mm	Solvent Control Length: _____ mm	
Minimum Concentration to Inhibit Growth (MCIG): 1 mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	7.0	3.0	Williams
LOEL	9.0	5.0	Williams
LC ₅₀	9.92	EC ₅₀ 6.62	
95% Confidence Limits 9.49-10.38		95% Confidence Limits 6.03-7.26	
TEST TERATOGENIC INDEX (TI = LC ₅₀ /EC ₅₀) = 1.498			

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%
2.5 mg/ml	____:____ X 100 = ____%	____:____ X 100 = ____%

FETAX SUMMARY SHEET

Test Material: P3D		Test No. Definitive #3
Source: Bottle		Investigator: Gillett/W./born
CAS No.	Lot No.	Laboratory: C-ERL
Composition / Purity:		Test Start Date: 11/19/93
Solvent: Conc.		Test End Date: 11/23/93
		Test Units: (i.e., mg/ml) mg/ml

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	6.94	7.0	7.0	7.0
Control	X	7.42	7.5	7.4	7.6
Highest Conc.	X	7.87	7.8	7.7	7.7

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	___:___ X 100 = 0 %	___:___ X 100 = 0 %
Solvent Control:	___:___ X 100 = ___ %	___:___ X 100 = ___ %
Control Length: 10.09 mm 10.09	Solvent Control Length: ___ mm	
Minimum Concentration to Inhibit Growth (MCIG): 3.0 mg/ml		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	7.0	Less than 0.25	Williams
LOEL	9.0	0.25	Williams
LC ₅₀ 7.56	EC ₅₀ 6.52		
95% Confidence Limits 7.18-7.97	95% Confidence Limits 5.9-7.21		

TEST TERATOGENIC INDEX (TI = LC ₅₀ /EC ₅₀) - 1.159

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	___:___ X 100 = ___ %	___:___ X 100 = ___ %
2.5 mg/ml	___:___ X 100 = ___ %	___:___ X 100 = ___ %

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>UNKNOWN P3D</u>		Investigator <u>TURLEY</u>
Source <u>OSU</u>		Lab <u>UMD/WREC</u>
CAS No.	Lot No.	Test Start Date <u>4/17/93</u>
Composition/Purity		Test End Date <u>4/21/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock <u>40 mg/ml</u>	8.53	8.41	8.55	8.60	8.51
Control	7.77	7.69	7.70	7.67	7.63
Highest Conc.	8.00	7.97	8.11	8.05	7.99

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number	<u>1</u> : <u>100</u> X 100 = <u>1</u> %	<u>6</u> : <u>99</u> X 100 = <u>6</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length <u>9.98</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>8.0</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>6.0</u>	FAIL HOMOGENEITY	Bonferroni T-test
LOEL	<u>7.0</u>	FAIL HOMOGENEITY	Bonferroni T-test
LC ₅₀	<u>7.76</u> (Trimmed Spearman-Kärber)	EC ₅₀ <u>7.92</u> mg/ml	Trimmed Spearman-Kärber
95% Confidence limits <u>7.49-8.04</u>		95% Confidence Limits <u>7.28-8.61</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>0.98</u>

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 4

Test Material <u>UNKNOWN B</u>		Investigator <u>TURLEY</u>
Source <u>OSU</u>		Lab <u>UMD/WREC</u>
CAS No.	Lot No.	Test Start Date <u>4/17/93</u>
Composition/Purity		Test End Date <u>4/21/93</u>
Solvent	Conc.	Test Units (l.p., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock	8.53	8.41	8.55	8.60	8.51
Control	7.77	7.69	7.70	7.67	7.63
Highest Conc.	8.00	7.97	8.11	8.05	7.99

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>1</u> : <u>100</u> X 100 = <u>1</u> %	<u>6</u> : <u>100</u> X 100 = <u>6</u> %
Solvent Control	_____ X 100 = _____ %	_____ X 100 = _____ %
Control Length <u>10.08</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>8.0</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>5.0</u>	<u>6.0</u>	Bonferroni T-test
LOEL	<u>6.0</u>	<u>7.0</u>	Bonferroni T-test
LC ₅₀ <u>7.67</u> mg/ml Trimmed Spearman-Kärber	EC ₅₀ <u>8.26</u> mg/ml Trimmed Spearman-Kärber		
95% Confidence limits <u>7.23 - 8.13</u>	95% Confidence Limits <u>7.84 - 8.71</u>		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 0.93

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ X 100 = _____ %	_____ X 100 = _____ %
2500 mg/L	_____ X 100 = _____ %	_____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 2

Test Material UNKNOWN P3D	Investigator TURLEY
Source OSU	Lab UMD/WREC
CAS No.	Lot No.
Composition/Purity	Test Start Date 3/2/93
Solvent	Conc.
	Test End Date 4/3/93
	Test Units (i.e., mg/ml) mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH					
Stock 40 mg/ml	8.48	8.41	8.51	8.43	8.55
Control	7.65	7.71	7.60	7.59	7.63
Highest Conc.	8.01	8.09	7.97	7.99	8.04

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	1 : 100 X 100 = 1 %	68 : 99 X 100 = 86 %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length 9.79 mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) 7.00		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	3.0	5.0	Bonferroni T-test
LOEL	4.0	6.0	Bonferroni T-test
LC ₅₀ 5.72 mg/ml	Trimmed Spearman-Kärber		
95% Confidence limits 5.42-6.02	EC ₅₀ 6.31 mg/ml Trimmed Spearman-Kärber		
	95% Confidence Limits 5.83-6.83		
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			0.91

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test Material <u>P3D</u>		Investigator <u>D.A. Dawson</u>
Source		Lab <u>WT/CUM</u>
CAS No.	Lot No.	Test Start Date <u>5/12/93</u>
Composition/Purity		Test End Date <u>5/16/93</u>
Solvent	Conc.	Test Units (l.o. mg/ml) <u>125/21</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	<u>7.44</u>	<u>7.41</u>	<u>7.44</u>	<u>7.43</u>	<u>—</u>
Control		<u>7.69</u>	<u>7.60</u>	<u>7.60</u>	<u>7.52</u>
Highest Conc.	<u>—</u>	<u>7.92 (9)</u>	<u>7.89 (7)</u>	<u>7.94 (7)</u>	<u>7.91 (7)</u>

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u>—</u> X 100 = %		
Total Number	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>3</u> : <u>100</u> X 100 = <u>3</u> %
Solvent Control	<u>—</u> : <u>—</u> X 100 = <u>—</u> %	<u>—</u> : <u>—</u> X 100 = <u>—</u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>5.</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>5.0</u> <u>WHL</u>	<u>5.0</u> <u>ASH</u>	
LOEL	<u>7.0</u> <u>WHL</u>	<u>7.0</u> <u>ASH</u>	
LC ₅₀	<u>12.32</u>	EC ₅₀ <u>8.77</u>	
95% Confidence limits (<u>11.7-12.8</u>)		95% Confidence Limits (<u>8.2-9.4</u>)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) (8.0) 1.40

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u>—</u> : <u>—</u> X 100 = <u>—</u> %	<u>—</u> : <u>—</u> X 100 = <u>—</u> %
2500 mg/L	<u>—</u> : <u>—</u> X 100 = <u>—</u> %	<u>—</u> : <u>—</u> X 100 = <u>—</u> %

D.A.D.

FETAX SUMMARY SHEET

Test Material <u>P3D</u>		Investigator <u>J. A. Dawson</u>
Source		Lab <u>UT/CVM</u>
CAS No.	Lot No.	Test Start Date <u>5/17/95</u>
Composition/Purity		Test End Date <u>5/21/95</u>
Solvent	Conc.	Test Units (i.e., mg/gl) <u>mg/ml</u>

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.41 <i>adj.</i>	7.44 <i>adj.</i>	7.42 <i>adj.</i>	7.45 <i>adj.</i>	—
Control	7.59	7.56	7.62	7.54	7.47
Highest Conc.	—	7.93	7.88	7.99	7.97

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	8.5 <i>both</i>	<5 <i>with</i>	
LOEL	10 <i>both</i>	5 <i>with</i>	
LC ₅₀ <u>11.68</u>	EC ₅₀ <u>9.23</u>		
95% Confidence limits (10.3 - 13.2)		95% Confidence Limits (7.1 - 12.1)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.27

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

J.A.D.

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>P3D</u>	Investigator <u>D.A. Dawson</u>
Source	Lab <u>WT/CMV</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>5/18/93</u>
Solvent	Test End Date <u>5/22/93</u>
Conc.	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>—</u> pH <u>—</u>					
Stock	7.41 <u>W</u>	7.41 <u>ad</u>	7.46 <u>ad</u>	7.44 <u>ad</u>	—
Control	7.56	7.59	7.47	7.46	7.42
Highest Conc.	—	7.18	7.74	8.04	7.93

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>1</u> : <u>100</u> X 100 = <u>1</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>7</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	8.5 <u>WDL</u>	5 <u>Bth</u>	
LOEL	10 <u>WDL</u>	7 <u>Bth</u>	
LC ₅₀ <u>11.96</u>		EC ₅₀ <u>8.21</u> <u>Q</u>	
95% Confidence limits (<u>11.7-12.3</u>)		95% Confidence Limits (<u>7.8-8.6</u>)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

1.46

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

D.A.D.
5/22/93

FETAX SUMMARY SHEET

Test Material <u>P3 D</u>		Investigator <u>Fort</u>
Source <u>QA/QC Corrections</u>		Lab <u>SP L</u>
CAS No.	Lot No.	Test Start Date <u>3/29/93</u>
Composition/Purity		Test End Date <u>4/2/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.4	7.5	7.5	
Control	7.9	7.9	8.0	7.9	
Highest Conc.	7.6	7.6	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>0</u> : <u>0</u> X 100 = <u>0</u> %	<u>1</u> : <u>100</u> X 100 = <u>1</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length <u>96.1</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>1.0</u>	<u>0.01</u>	<u>Williams</u>
LOEL	<u>5.0</u>	<u>0.05</u>	
LC ₅₀	<u>8.91</u>	EC ₅₀	<u>1.55</u>
95% Confidence limits <u>7.68 - 10.35</u>		95% Confidence Limits <u>1.20 - 1.99</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 5.75

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>P3D</u>	Investigator <u>Fort</u>
Source <u>QA/QC Corrections</u>	Lab <u>SBL</u>
CAS No. _____ Lot No. _____	Test Start Date <u>4/26/93</u>
Composition/Purity _____	Test End Date <u>4/30/93</u>
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	<u>7.2</u>	<u>7.2</u>	<u>7.3</u>	<u>7.2</u>	
Control	<u>7.9</u>	<u>7.9</u>	<u>7.8</u>	<u>7.9</u>	
Highest Conc.	<u>7.5</u>	<u>7.5</u>	<u>7.4</u>	<u>7.4</u>	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed _____ X 100 = %		
Total Number _____	<u>1</u> : <u>100</u> X 100 = <u>1</u> %	<u>3</u> : <u>99</u> X 100 = <u>3</u> %
Solvent Control _____	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length <u>98.9</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>7.5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.75</u>	<u>1.0</u>	<u>Williams Test</u>
LOEL	<u>1.0</u>	<u>2.5</u>	
LC ₅₀	<u>7.31</u>	EC ₅₀	<u>2.30</u>
95% Confidence limits	<u>6.39-8.36</u>	95% Confidence Limits	<u>1.96-2.69</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 3.18

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3D	Investigator	Fort
Source	QA/QC Corrections	Lab	SBL
CAS No.	Rescored	Test Start Date	4/26/93
Composition/Purity		Test End Date	4/30/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.4	7.5	7.5	
Control	7.9	7.9	7.9	8.0	
Highest Conc.	7.7	7.6	7.6	7.6	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	2 : 100 X 100 = 2%	0 : 98 X 100 = 0%
Solvent Control	: X 100 = %	: X 100 = %
Control Length 98.0 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 2.5		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.0	0.75	Williams Test
LOEL	2.5	1.0	
LC ₅₀	5.46	EC ₅₀	1.08
95% Confidence limits	4.63 - 6.43	95% Confidence Limits	0.99 - 1.19
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			5.06

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

SODIUM ARSENATE

ORIGINAL DATA TABLE WITH MATERIALS

units are in mg/ml

	LAB	LC50	CI LO	CI HI	EC50	CI LO	CI HI	EC50	CI LO	CI HI	MCIG	TI	LOEC/MORT	LOEC/MALF	SUBTOTALS:	STD:	CV:
BANTLE/ RAYBURN	1 1	2.53	1.79	3.59	0.58	0.43	0.80	0.30	4.4	1.50	0.30	LC50	2.09	0.311	14.9		
	2	1.87	1.31	2.69	0.21	0.14	0.33	0.10	8.9	1.50	0.30	EC50	0.40	0.151	38.1		
	3	1.87	1.33	2.63	0.40	0.26	0.61	1.00	4.7	2.00	0.30	MCIG	0.47	0.386	82.7		
												TI	5.27	2.072	39.3		
BANTLE/ HULL	1 2	2.65	2.06	3.41	0.59	0.47	0.74	1.00	4.5	1.25	0.50	LC50	3.03	0.442	14.6		
	2	3.65	3.12	4.28	0.45	0.35	0.58	1.00	8.1	4.00	0.50	EC50	0.43	0.140	32.4		
	3	2.79	2.23	3.48	0.25	0.16	0.38	1.00	11.2	1.50	0.08	MCIG	1.00	0.000	0.0		
												TI	7.05	2.726	38.7		
FINCH	1 3	1.53	1.31	1.78	1.27	0.98	1.64	1.50	1.2	1.00	1.00	LC50	2.07	0.454	22.0		
	2	2.03	1.75	2.35	1.91	1.64	2.22	1.00	1.1	1.00	2.00	EC50	1.40	0.378	27.1		
	3	2.64	2.36	2.95	1.01	0.82	1.25	1.00	2.6	1.50	0.50	MCIG	1.17	0.236	20.2		
												TI	1.48	0.700	47.3		
LINDER/ BUCHWALTER	1 4											LC50	#DIV/0!	#DIV/0!	#DIV/0!		
	2											EC50	#DIV/0!	#DIV/0!	#DIV/0!		
	3											MCIG	#DIV/0!	#DIV/0!	#DIV/0!		
												TI	#DIV/0!	#DIV/0!	#DIV/0!		
BURTON/ TURLEY	1 5	1.77	1.61	1.94	1.23	1.03	1.46	1.50	1.4	1.50	1.00	LC50	2.07	0.293	14.1		
	2	2.47	2.31	2.65	1.54	1.35	1.74	1.50	1.6		1.50	EC50	1.28	0.192	14.9		
	3	1.98	1.82	2.15	1.08	0.90	1.30	1.50	1.8	1.50	1.00	MCIG	1.50	0.000	0.0		
												TI	1.62	0.162	10.0		
DAWSON	1 6	2.89	2.69	3.09	2.01	1.86	2.17	1.00	1.4	2.00	1.50	LC50	2.45	0.377	15.4		
	2	2.50	2.34	2.67	1.64	1.49	1.80	1.00	1.5	2.00	1.50	EC50	1.76	0.174	9.9		
	3	1.97	1.83	2.13	1.64	1.50	1.81	1.00	1.2	1.00	1.00	MCIG	1.00	0.000	0.0		
												TI	1.39	0.137	9.8		
STOVER/ FORT	1 7	1.89	1.62	2.20	0.39	0.31	0.49	1.00	4.8	1.00	0.10	LC50	2.08	0.138	6.6		
	2	2.16	1.83	2.56	0.56	0.49	0.64	2.50	3.9	0.75	0.50	EC50	0.52	0.097	18.6		
	3	2.20	1.83	2.65	0.62	0.54	0.70	1.00	3.5	0.75	0.25	MCIG	1.50	0.707	47.1		
												TI	3.98	5.54E-01	13.9		
GRAND TOTAL																	

Table 5. Results of the Interlaboratory Study for Sodium Arsenate.

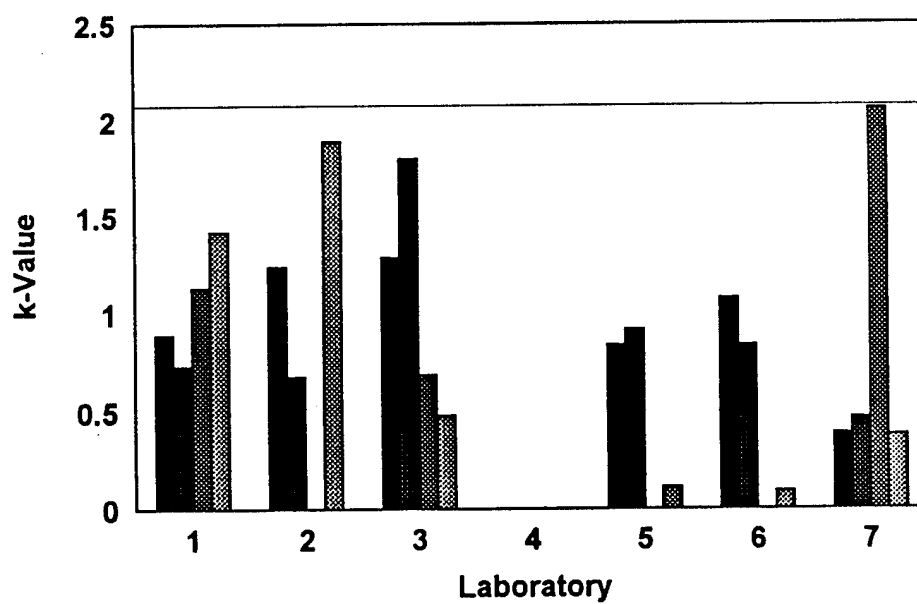
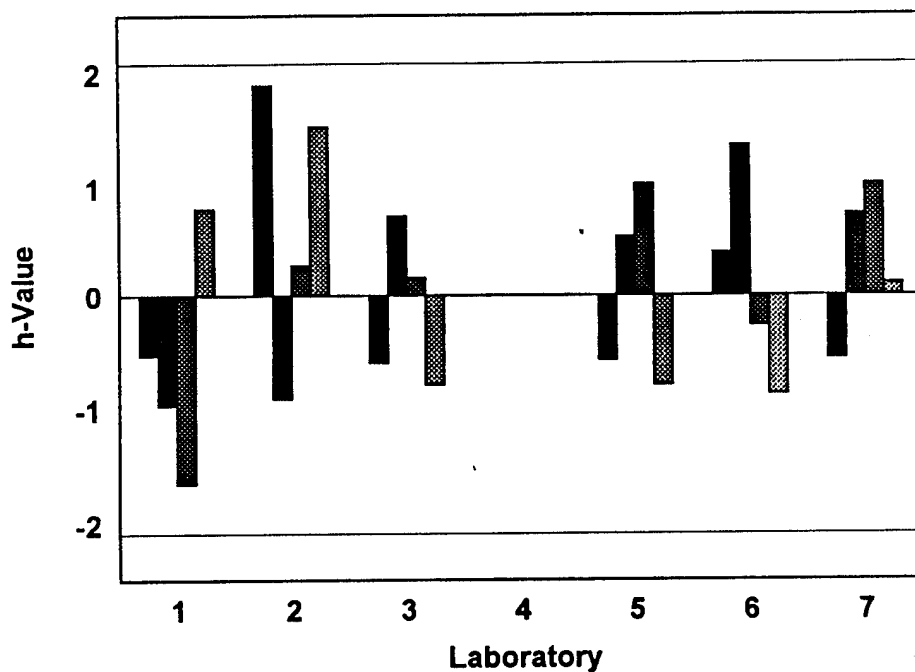
Laboratory	Replicate (n)	Mean LC50		Mean EC50		Mean TI ²		Mean MCIG ³		Mean CV	
		LC50 (mg/ml)	CV ¹ (%)	EC50 (mg/ml)	CV (%)	TI ²	CV (%)	MCIG ³ (mg/ml)	CV (%)	MCIG (mg/ml)	CV (%)
1	1	2.53		0.58				0.30			
	2	1.87	14.9	0.21	38.1	5.3	39.3	0.10	82.7	0.47	
	3	1.87		0.40				1.00			
2	1	2.65		0.59				1.00			
	2	3.65	14.6	0.45	32.4	7.0	38.7	1.00	0.0	1.00	
	3	2.79		0.25				1.00			
3	1	1.53		1.27				1.50			
	2	2.03	22.0	1.91	27.1	1.5	47.3	1.00	20.2	1.17	
	3	2.64		1.01				1.00			
4	1										
	2										
	3										
5	1	1.77		1.23				1.50			
	2	2.47	14.1	1.54	14.9	1.6	10.0	1.50	0.0	1.50	
	3	1.98		1.08				1.50			
6	1	2.89		2.01				1.00			
	2	2.50	15.4	1.64	9.9	1.4	9.8	1.00	0.0	1.00	
	3	1.97		1.64				1.00			
7	1	1.89		0.39				1.00			
	2	2.16	6.6	0.56	18.6	4.0	13.9	2.50	47.1	1.50	
	3	2.20		0.62				1.00			

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of Sodium Arsenate



FETAX Summary Sheet

Test No. DEF 1

Test Material	P3E	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	3-25-93
Composition/Purity		Test End Date	3-29-93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.6	7.6	7.8	7.8	
Control		7.8	7.5	7.3	7.1
Highest Concentration		7.6	7.3	7.2	7.1

No. Dead or Malformed		
_____ X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	10 : 100 X 100 = 10%	6 : 90 X 100 = 6.7%
Solvent Control	: X 100 =	: X 100 =
Control Length	e20 9.27	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG) 0.3 mg/ml J2+		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	0.1	T-test
LOEL	1.5	0.3	T-test
LC50	2.531	EC50	0.584
95% CL	1.786 -- 3.586	95% Confidence limits	0.427 ---- 0.798
Test Teratogenic Index (TI = LC50/EC50): 4.34 4.33			
95% Confidence limits 2.71 -- 6.93			

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test Material		P3E	Investigator		JAMES RAYBURN
Source		OAK RIDGE	Laboratory		BANTLE
CAS No.	Lot No.		Test Start Date		APRIL 26 1993
Composition/Purity			Test End Date		APRIL 30 1993
Solvent	Conc.		Test Units (i.e., mg/ml)		MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.8	8	7.9	7.7	
Control		7.3	7.7	7.7	7.8
Highest Concentration		7.5	7.2	7.4	6.9

No. Dead or Malformed
_____ X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

10 : 100	X 100 =	10%	7 : 90	X 100 =	7.8%
G19 : E19	X 100 =	G19	H19 : J19	X 100 =	L19

G20 8.75

Solvent Control Length

0.1

42+

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	0.1	T-test
LOEL	1.5	0.3	T-test
LC50	1.874	EC50	0.212
95% CL	1.307 -- 2.687	95% Confidence limits	0.135 ---- 0.331
Test Teratogenic Index (TI = LC50/EC50):			8.86
95% Confidence limits			4.99 -- 15.74

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	X 100 =	X 100 =
2500 mg/L	X 100 =	X 100 =

CL= Confidence limits

FETAX Summary Sheet

Test No. 3

Test Material	P3E	Investigator	JAMES RAYBURN
Source	OAK RIDGE	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	APRIL 26 1993
Composition/Purity		Test End Date	APRIL 30 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	E11	F11	G11	J11	
Control		F12	G12	J11	K12
Highest Concentration		F13	B13	J11	K13

No. Dead or Malformed
_____ X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

9 : 100 X 100 = 9% 9 : 91 X 100 = 9.9%

G19 : E19 X 100 = G19 H19 : J19 X 100 = L19

Control Length 8.5 Solvent Control Length J20

10/1/1 det

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.01	0.1	T-test
LOEL	2	0.3	T-test
LC50	1.869	EC50	0.402
95% CL	1.330 -- 2.628	95% Confidence limits	0.263 ---- 0.613

Test Teratogenic Index (TI = LC50/EC50): 4.65

95% Confidence limits 2.70 -- 8.00

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	X 100 =	: X 100 =
2500 mg/L	X 100 =	: X 100 =

CL= Confidence limits

FETAX Summary Sheet

Test No. 1

Test Material	P3E	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU
CAS No.	Lot No.	Test Start Date:	Mar 15 1993
Composition/Purity		Test End Date	Mar 19 1993
Solvent	Conc.	Test Units (l.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	8.1	8.0	8.2	8.0	X
Control	X	7.7	7.6	7.6	7.4
Highest Concentration	X	8.0	7.8	7.5	7.2

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMIT

Total Number	Mortality Record		Malformation Record	
FETAX Control	4 : 100	X 100 = 4%	10 : 96	X 100 = 10.4%
Solvent Control	:	X 100 =	:	X 100 =
Control Length	0.9	cm	Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)			1.0 mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.25	T-test
LOEL	1.25	0.5	T-test
LC50	2.648	EC50 0.590	
95% CL	2.055 -- 3.412	95% Confidence limits 0.472 ---- 0.736	

Test Teratogenic Index (TI = LC50/EC50): 4.49

95% Confidence limits 3.21 -- 6.29

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 2

Test Material	P3E	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU
CAS No.	Lot No.	Test Start Date:	Jun 14 1993
Composition/Purity		Test End Date	Jun 18 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	8.0	8.0	8.1	8.0	X
Control	X	7.8	7.6	7.7	7.6
Highest Concentration	X	8.0	7.7	7.6	7.3

No. Dead or Malformed

_____ X 100 = %

MALFORMATION EXCEED ASTM LIMIT

Total Number	Mortality Record		Malformation Record	
FETAX Control	7 : 100	X 100 = 7%	10 : 93	X 100 = 10.8%
Solvent Control	:	X 100 =	:	X 100 =
Control Length	1 cm	Solvent Control Length		
Minimum Concentration to Inhibit Growth (MCIG)		1.0 mg/ml		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	2	0.25	T-test
LOEL	4	0.5	T-test
LC50	3.654	EC50 0.448	
95% CL	3.124 -- 4.275	95% Confidence limits 0.345 ---- 0.581	

Test Teratogenic Index (TI = LC50/EC50):	8.16
95% Confidence limits	6.02 -- 11.06

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality		Malformation	
5.5 mg/L	C33 : E33	X 100 = G33	H33 : J33	X 100 = L33
2500 mg/L	C34 : E34	X 100 = G34	H33 : J34	X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 3

Test Material	P3E	Investigator	Mendi A. Hull
Source		Laboratory	Bantle / OSU
CAS No.	Lot No.	Test Start Date:	Mar 15 1993
Composition/Purity		Test End Date	Mar 19 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	8.0	8.1	8.0	8.0	X
	Control	X	7.5	7.5	7.8	7.4
	Highest Concentration	X	8.0	7.7	7.9	7.6

No. Dead or Malformed				
	X 100 = %			
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 100	X 100 = 0%	10 : 100	X 100 = 10.0%
Solvent Control	:	X 100 =	:	X 100 =
Control Length	1 cm	Solvent Control Length		
Minimum Concentration to Inhibit Growth (MCIG)		1.0 mg/ml		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	N.A.	T-test
LOEL	1.5	0.075	T-test
LC50	2.785	EC50	0.247
95% CL	2.230 -- 3.479	95% Confidence limits	0.162 ---- 0.376

Test Teratogenic Index (TI = LC50/EC50): 11.29

95% Confidence limits 7.00 -- 18.21

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	C33 : E33 X 100 = G33	H33 : J33 X 100 = L33
2500 mg/L	C34 : E34 X 100 = G34	H33 : J34 X 100 = L34

CL= Confidence limits

FETAX Summary Sheet

Test No. 1(201-01)

Test Material	P3E	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	Lot No.	Test Start Date:	12 JULY 93
Composition/Purity		Test End Date	16 JULY 93
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	8.9	7.8	7.6	7.6	
	Control		7.6	7.7	7.7	7.7
	Highest Concentration		7.6	7.3	7.4	7.4

No. Dead or Malformed	
----- X 100 = %	
Total Number	
FETAX Control	1 : 100 X 100 = 1% ✓
Solvent Control	X 100 = -
Control Length	9.715
Solvent Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	1.5 ✓

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.5	T-test
LOEL	1	1	T-test
LC50	1.526 ✓	EC50	1.271 ✓
95% CL	1.310 -- 1.778	95% Confidence limits	0.983 --- 1.642
Test Teratogenic Index (TI = LC50/EC50):			1.20 ✓
95% Confidence limits			0.89 -- 1.62

CL = Confidence limits

FETAX Summary Sheet

Test No. 2(201-02)

Test Material	P3E	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Test Start Date:	12 JULY 93
Composition/Purity	-	Test End Date	16 JULY 93
Solvent	-	Test Units (i.e., mg/ml)	MG/ML

		Day 1	Day 2	Day 3	Day 4
pH	Stock	8.0	7.6	7.6	7.6
	Control	-	7.8	7.7	7.7
	Highest Concentration	-	7.6	7.3	7.4

No. Dead or Malformed			
_____ X 100 = %			
Total Number			
FETAX Control	Mortality Record	Malformation Record	
	3 : 100 X 100 = 3%	7 : 97 X 100 = 7.2%	
Solvent Control	- X 100 = -	- X 100 = -	
Control Length	9.754	Solvent Control Length	-
Minimum Concentration to Inhibit Growth (MCIG)		1.0	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	1.5	T-test
LOEL	1	2	T-test
LC50	2.030 ? ~1.8?	EC50 1.907 ? ~2.2?	
95% CL	1.752 - 2.352	95% Confidence limits 1.636 - 2.221	

Test Teratogenic Index (TI = LC50/EC50): ~0.8? (1.06) ?

95% Confidence limits 0.86 - 1.32

CL = Confidence limits

FETAX Summary Sheet

Test No. 3(201-03)

Test Material	P3E	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	19 JUL 93
Solvent	-	Test End Date	23 JUL 93
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.9	7.8	7.9	7.8	
Control		7.7	7.6	7.6	7.7
Highest Concentration		7.7	7.6	7.6	7.6

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	1 : 100 X 100 = <input checked="" type="checkbox"/> 1%	6 : 99 X 100 = 6.1%
Solvent Control	X 100 = -	X 100 = -
Control Length	9.839	Solvent Control Length
Minimum Concentration to Inhibit Growth (MCIG)	1.0	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	N.A.	T-test
LOEL	1.5	0.5	T-test
LC50	2.640	2.5	EC50 1.014
95% CL	2.361	2.952	95% Confidence limits 0.824 --- 1.250
Test Teratogenic Index (TI = LC50/EC50):			~1.4? 2.60
95% Confidence limits			2.05 -- 3.30

CL= Confidence limits

FETA SUMMARY SHEET

Test No. 1

Test Material <u>UNKNOWN P3E</u>	Investigator <u>TURLEY</u>
Source <u>OSU</u>	Lab <u>UMD./WREC</u>
CAS No. _____ Lot No. _____	Test Start Date <u>5/20/93</u>
Composition/Purity _____	Test End Date <u>5/24/93</u>
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	9.16	9.10	9.07	9.13	9.15
Control	7.61	7.57	7.60	7.55	7.60
Highest Conc.	8.48	8.40	8.38	8.44	8.46

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = % Total Number	<u>2</u> : <u>100</u> X 100 = <u>2</u> %	<u>4</u> : <u>98</u> X 100 = <u>4</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length <u>10.16</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.5</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>1.0</u> mg/ml	<u>0.5</u> mg/ml	Bonferroni T-test
LOEL	<u>1.5</u> mg/ml	<u>1.0</u> mg/ml	Bonferroni T-test
LC ₅₀ <u>1.77</u> mg/ml (Trimmed Spearman-Kärber)	EC ₅₀ <u>1.23</u> mg/ml (Trimmed Spearman-Kärber)		
95% Confidence limits (<u>1.61</u> - <u>1.94</u>)	95% Confidence Limits (<u>1.03</u> - <u>1.46</u>)		
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>1.44</u>

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
<u>5.5</u> mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
<u>2500</u> mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAL SUMMARY SHEET

Test No. 2

Test Material <u>UNKNOWN P3E</u>		Investigator <u>TURLEY</u>
Source <u>O.S.U</u>		Lab <u>UMD/WREC</u>
CAS No.	Lot No.	Test Start Date <u>June 15, 1993</u>
Composition/Purity		Test End Date <u>June 19, 1993</u>
Solvent	Conc.	Test Units (i.e., mg/ml)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	9.13	9.14	9.15	9.09	9.06
Control	7.59	7.55	7.61	7.55	7.49
Highest Conc. (50)	8.31	8.44	8.39	8.33	8.27

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
	$2 : 100 \times 100 = 2\%$	$6 : 98 \times 100 = 6\%$
Solvent Control		
Control Length <u>9.89</u> mm		
Minimum Concentration to Inhibit Growth (MCIG) <u>1.5</u> mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	Fail Normality/Homogeneity	1.0 mg/ml	Bonferroni T-test
LOEL	Fail Normality/Homogeneity	1.5 mg/ml	Bonferroni T-test
LC ₅₀ <u>2.47</u> mg/ml (Trimmed Spearman-Kärber)		EC ₅₀ <u>1.54</u> mg/ml (Trimmed Spearman-Kärber)	
95% Confidence limits <u>2.31-2.65</u>		95% Confidence Limits <u>1.35-1.74</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.60

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L		
2500 mg/L		

FETAL SUMMARY SHEET

Test No. 3

Test Material UNKNOWN P3E		Investigator TURLEY
Source O.S.U.		Lab UMD/WREC
CAS No.	Lot No.	Test Start Date June 24, 1993
Composition/Purity		Test End Date June 28, 1993
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	9.11	9.19	9.09	9.13	9.14
Control	7.55	7.59	7.61	7.57	7.53
Highest Conc. 50/mg/L	8.40	8.43	8.40	8.42	8.38

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number. X 100 = %	2 : 100 X 100 = 2 %	8 : 98 X 100 = 8 %
Solvent Control	: X 100 = %	: X 100 = %
Control Length 10.02 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 1.5 mg/ml		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.0 mg/ml	0.5 mg/ml	Benferroni T-test
LOEL	1.5 mg/ml	1.0 mg/ml	Benferroni - T-test
LC ₅₀	1.98 (Trimmed Spearman-Kärber)	EC ₅₀ 1.08 (Trimmed Spearman-Kärber)	
95% Confidence limits	1.82 - 2.15	95% Confidence Limits (0.90 - 1.30)	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			1.83

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

FETAX SUMMARY SHEET

Test No. 1

Test Material <u>P3E</u>		Investigator <u>D.A. DAWSON</u>
Source		Lab <u>UT/UCM</u>
CAS No.	Lot No.	Test Start Date <u>5/3/93</u>
Composition/Purity		Test End Date <u>5/7/93</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

<u>— pH —</u>	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.39 <i>adj.</i>	7.40 <i>adj.</i>	7.41 <i>adj.</i>	7.41 <i>adj.</i>	—
Control	7.74	7.63	7.52	7.57	7.61
Highest Conc.	—	7.59	7.61	7.67	7.71

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u>0</u> X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>2</u> : <u>100</u> X 100 = <u>2</u> %
Total Number		
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.0</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.5 both	1.0 both	
LOEL	2.0 both	1.5 both	
LC ₅₀ 2.89	L&W	EC ₅₀ 2.01	L&W
95% Confidence limits 2.09 - 3.09		95% Confidence Limits (1.86 - 2.17)	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			1.44

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <i>not</i>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L <i>done</i>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

D.A.D.

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>P3E</u>	Investigator <u>D.A. Dawson</u>
Source	Lab <u>WT/CVM</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>5/10/93</u>
Solvent	Test End Date <u>5/14/93</u>
Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u> </u> pH <u> </u>					
Stock	7.37 adj	7.38 adj	7.39 adj	7.40 adj	—
Control	7.72	7.61	7.66	7.46	7.45
Highest Conc.	—	7.60	7.56	7.56	7.52

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
<u> </u> X 100 = %		
Total Number	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>3</u> : <u>100</u> X 100 = <u>3</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.0</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.5 both	1.0 both	
LOEL	2.0 both	1.5 both	
LC ₅₀ 2.55	LC ₅₀	EC ₅₀ 1.64	LC ₅₀
95% Confidence limits (2.34 - 2.67)		95% Confidence Limits (1.49 - 1.80)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

1.52

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <u>not</u>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L <u>done</u>	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

D.A.D

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3E	Investigator	D.A. DAWSON
Source		Lab	WT/CVM
CAS No.		Lot No.	
Composition/Purity		Test Start Date	5/11/93
Solvent		Test End Date	5/15/93
Conc.		Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.38 <i>adj.</i>	7.39 <i>adj.</i>	7.41 <i>adj.</i>	7.39 <i>adj.</i>	--
Control	7.70	7.60	7.52	7.43	7.39
Highest Conc.	—	7.61	7.55	7.59	7.54

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	0 : 100 X 100 = 0 %	3 : 100 X 100 = 3 %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 1.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	< 1.0 <i>WIL.</i>	< 1.0 <i>BOTH</i>	
LOEL	1.0 <i>WIL.</i>	1.0 <i>BOTH</i>	
LC ₅₀ 1.97	EC ₅₀ 1.64		
95% Confidence limits (1.83 - 2.13)		95% Confidence Limits (1.50 - 1.81)	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 1.20

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L <i>not</i>	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L <i>done</i>	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test Material P3E		Investigator Fort
Source QA/QC Corrections		Lab SBL
CAS No.	Lot No.	Test Start Date 3/29/93
Composition/Purity		Test End Date 4/2/93
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.6	7.4	7.3	
Control	8.0	8.1	8.0	8.0	
Highest Conc.	7.5	7.7	7.7	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>0</u> : <u>0</u> X 100 = <u>0</u> %	<u>0</u> : <u>0</u> X 100 = <u>0</u> %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length 96.3 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 1.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.5	0.05	Williams Test
LOEL	1.0	0.1	
LC ₅₀	(PF) 0.76	(1.89)	EC ₅₀ 0.39
95% Confidence limits 0.72 - 0.81		95% Confidence Limits 0.31 - 0.49	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			4.85

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>P3E</u>	Investigator <u>Fort</u>
Source <u>QA/QC corrections</u>	Lab <u>SBL</u>
CAS No. <u>Resorcinol</u> Lot No. _____	Test Start Date <u>4/26/93</u>
Composition/Purity _____	Test End Date <u>4/30/93</u>
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	7.4	7.3	7.2	7.2	
Control	7.9	7.9	7.8	7.9	
Highest Conc.	7.5	7.4	7.5	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed _____ X 100 = %		
Total Number _____	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>4</u> : <u>100</u> X 100 = <u>4</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length <u>97.8</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>2.5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.5</u>	<u>0.25</u>	<u>Williams test</u>
LOEL	<u>0.75</u>	<u>0.5</u>	
LC ₅₀	<u>2.16</u>	EC ₅₀ <u>0.56</u>	
95% Confidence limits	<u>1.83 - 2.56</u>	95% Confidence Limits <u>0.49 - 0.64</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>3.86</u>

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3E	Investigator	Fort
Source	QA/QC Correction	Lab	SBL
CAS No.	Rescored	Test Start Date	4/26/93
Lot No.		Test End Date	4/30/93
Composition/Purity		Test Units (i.e., mg/ml)	mg/ml
Solvent	Conc.		

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.4	7.3	7.2	7.2	
Control	7.8	7.9	7.8	7.9	
Highest Conc.	7.4	7.5	7.5	7.5	

FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed	X 100 = %				
Total Number		0 : 100 X 100 = 0 %		0 : 100 X 100 = 0 %	
Solvent Control		: X 100 = %		: X 100 = %	
Control Length	97.1 mm	Solvent Control Length		mm	
Minimum Concentration to Inhibit Growth (MCIG)		1.0			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.5	0.1	Williams Test
LOEL	0.75	0.25	
LC ₅₀	2.20	EC ₅₀	0.62
95% Confidence limits	1.83 - 2.65	95% Confidence Limits	0.54 - 0.70

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

3.55

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

COPPER SULFATE

PHASE III COMPOUND F DATA (P3F) CuSO(4)

FETAX ILS STUDY: 7 LABS, 8 LEVELS, 3 REPLICATES
 units are in mg/L

PAGE 1
 ORIGINAL DATA TABLE WITH MATERIALS

	LAB	units are in mg/L										SUBTOTALS:	STD:	CV:	
		LC50 A	CI LO B	CI HI C	EC50 D	CI LO E	CI HI F	MCIG G	TI H	LOEC/MORT I	LOEC/MALF J				
BANTLE/ RAYBURN	1	1.00	0.89	1.11	0.32	0.28	0.37	0.70	3.1	0.90	0.20	LC50	1.56	0.513	32.8
	2	1.45	1.16	1.81	0.21	0.15	0.28	0.40	6.9	2.00	0.20	EC50	0.28	0.050	17.7
	3	2.24	1.72	2.91	0.31	0.28	0.35	0.60	7.2	1.00	0.20	MCIG TI	0.57 5.58	0.125 1.862	22.0 33.4
BANTLE/ HULL	1	0.63	0.48	0.84	0.15	0.12	0.19	0.20	4.2	0.60	0.20	LC50	0.77	0.107	13.9
	2	0.79	0.61	1.03	0.24	0.18	0.33	0.40	3.3	0.60	0.20	EC50	0.20	0.039	19.0
	3	0.89	0.68	1.15	0.22	0.16	0.30	0.20	4.0	4.00	0.10	MCIG TI	0.27 3.79	0.094 0.397	35.4 10.5
FINCH	1	0.93	0.73	1.20	0.33	0.21	0.54	0.05	2.0			LC50	0.85	0.132	15.6
	2	0.66	0.52	0.85	0.56	0.47	0.66	0.05	1.7			EC50	0.45	0.115	25.8
	3	0.95	0.95	1.39				0.01				MCIG TI	0.04 1.90	0.019 0.152	51.4 8.0
LINDER/ BUCHWALTER	1	0.29	0.22	0.38	0.08	0.05	0.12	0.01	3.6	0.50	0.03	LC50	0.46	0.122	26.8
	2	0.50	0.38	0.65	0.11	0.06	0.19	0.03	4.5	0.50	0.08	EC50	0.20	0.149	74.5
	3	0.58	0.51	0.66	0.41	0.19	0.86	0.08	1.4	0.50	0.10	MCIG TI	0.04 2.28	0.029 1.314	84.1 57.5
BURTON/ TURLEY	1	1.70	1.34	2.15	2.47	0.32	19.30	0.75	0.7	0.75	1.00	LC50	2.17	0.430	19.8
	2	2.07	1.77	2.41	1.61	1.21	2.14	1.00	1.3		3.00	EC50	2.68	0.966	36.1
	3	2.74	1.97	3.65	3.95	3.13	4.99	1.00	0.7	2.00	3.00	MCIG TI	0.92 0.81	0.118 0.280	12.9 34.6
DAWSON	1	0.88	0.79	0.99	0.81	0.72	0.91	0.25	1.1	0.38	0.75	LC50	0.88	0.045	5.1
	2	0.82	0.74	0.90	0.76	0.68	0.86	0.25	1.1	0.63	0.75	EC50	0.79	0.022	2.7
	3	0.93	0.83	1.05	0.80	0.71	0.91	0.10	1.2	0.63	0.50	MCIG TI	0.20 1.11	0.071 0.038	35.4 3.4
STOVER/ FORT	1	1.49	1.31	1.70	1.48	1.18	1.85	0.50	1.0			LC50	1.93	0.312	16.2
	2	2.09	2.00	2.18	0.89	0.85	0.94	0.30	2.3			EC50	1.10	0.271	24.7
	3	2.20	2.10	2.51	0.92	0.84	1.03	0.50	2.4			MCIG TI	0.43 1.76	0.094 0.643	21.8 36.6

GRAND TOTAL	LAB	units are in mg/L								SUBTOTALS:	STD:	CV:	
		LC50 A	CI LO B	CI HI C	EC50 D	CI LO E	CI HI F	MCIG G	TI H	LOEC (mort) I	LOEC (malif) J		
	1	1.23	0.67	0.99	0.81	0.72	0.91	0.25	1.1	0.38	0.75	0.88	5.1
	2	0.83	0.9260	0.90	0.76	0.68	0.86	0.25	1.1	0.63	0.75	0.79	2.7
	3	0.35	0.31	1.05	0.80	0.71	0.91	0.10	1.2	0.63	0.50	0.20	35.4
												1.11	3.4
												1.93	16.2
												0.271	24.7
												0.43	21.8
												1.76	36.6

Table 6. Results of the Interlaboratory Study for Copper Sulfate.

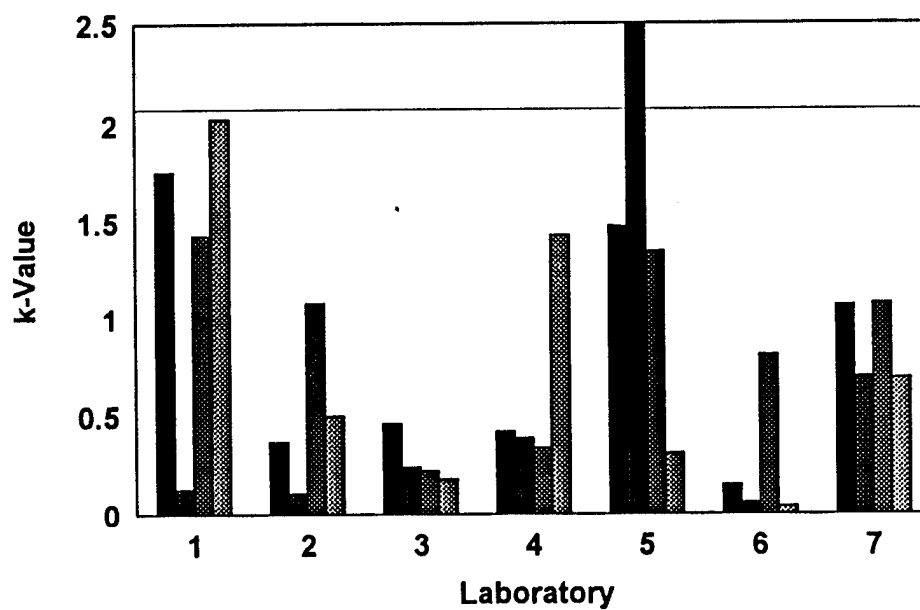
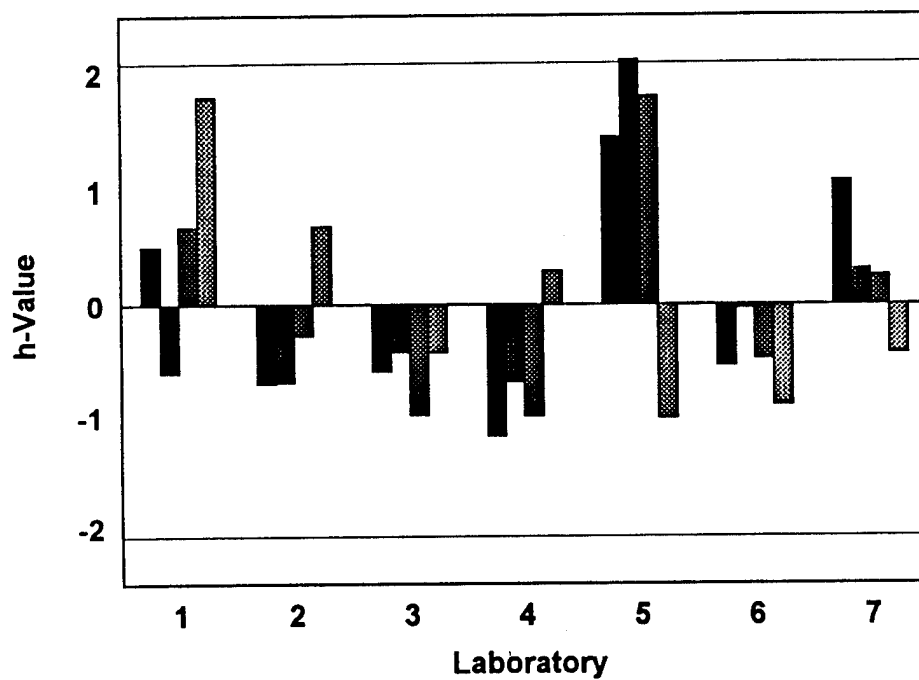
Laboratory	Replicate (n)	Mean LC50		Mean EC50		Mean TI ²		TI CV		Mean MCIG ³		Mean MCIG CV	
		LC50 (mg/L)	CV1 (%)	EC50 (mg/L)	CV (%)	EC50 (mg/L)	CV (%)	TI ²	(%)	MCIG ³ (mg/L)	(mg/L)	MCIG (mg/L)	CV (%)
1	1	1.00		0.32						0.70			
	2	1.45	32.8	0.21	17.7	0.28	33.4	5.6		0.40	0.57	22.0	
	3	2.24		0.31						0.60			
2	1	0.63		0.15						0.20			
	2	0.79	13.9	0.24	19.0	0.20	10.5	3.8		0.40	0.27	35.4	
	3	0.89		0.22						0.20			
3	1	0.93		--						0.05			
	2	0.66	15.6	0.33	25.8	0.45	8.0	1.9		0.05	0.04	51.4	
	3	0.95		0.56						0.01			
4	1	0.29		0.08						0.01			
	2	0.50	26.8	0.11	74.5	0.20	57.5	2.3		0.03	0.04	84.1	
	3	0.58		0.41						0.08			
5	1	1.70		2.47						0.75			
	2	2.07	19.8	1.61	36.1	2.68	34.6	0.8		1.00	0.92	12.9	
	3	2.74		3.95						1.00			
6	1	0.88		0.81						0.25			
	2	0.82	5.1	0.76	2.7	0.79	3.4	1.1		0.25	0.20	35.4	
	3	0.93		0.80						0.10			
7	1	1.49		1.48						0.50			
	2	2.09	16.2	0.89	24.7	1.10	36.6	1.8		0.30	0.43	21.8	
	3	2.20		0.92						0.50			

¹CV=Coefficient of Variation.

²TI=Mean Teratogenic Index calculated by dividing the Mean LC50 by the Mean EC50 for each laboratory.

³MCIG=Minimum Concentration to Inhibit Growth.

ASTM Analysis of Copper Sulfate



FETAX Summary Sheet

Test No. DEF. 1

Test Material	P3F	Investigator	JAMES RAYBURN
Source		Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	APRIL 25 1994
Composition/Purity		Test End Date	APRIL 29 1994
Solvent	NONE	Conc.	Test Units (i.e., mg/ml) MG/L

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.8	6.7	6.8	6.8	
Control		7.7	7.7	7.7	7.3
Highest Concentration		6.8	7	7	7.1

No. Dead or Malformed		
X 100 = %		
Total Number		
	Mortality Record	Malformation Record
FETAX Control	3 : 100 X 100 = 3%	8 : 97 X 100 = 8.2%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	8.7	Solvent Control Length (mm)
Minimum Concentration to Inhibit Growth (MCIG)	0.7	MG/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.3	0.1	T-test
LOEL	16 0.9	0.2	T-test
LC50	0.995	EC50	0.317
95% CL	0.894 -- 1.107	95% Confidence limits	0.275 ---- 0.365
Test Teratogenic Index (TI = LC50/EC50):		3.14	
95% Confidence limits		2.63 -- 3.75	

FETAX Summary Sheet

Test No. 2

Test Material	P3F	Investigator	James Rayburn <i>JRB</i>
Source		Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	09-JUN-94
Composition/Purity		Test End Date	12-JUN-94
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/l

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7	7	7	7.1	
Control		7.5	7.6	7.1	7.4
Highest Concentration		7.5	7.3	7.2	7.3

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.7

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

4 : 100

X 100 =

4%

10 : 96

X 100 =

10.4%

X 100 =

X 100 =

Solvent Control Length (mm)

0.4

mg/l

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	0.1	T-test
LOEL	2	0.2	T-test
LC50	1.448	EC50	0.206
95% CL	1.160 -- 1.808	95% Confidence limits	0.151 ---- 0.282

Test Teratogenic Index (TI = LC50/EC50):

7.03

95% Confidence limits

4.79

--

10.32

FETAX Summary Sheet

Test No. 3

Test Material	P3F	Investigator	James Rayburn <i>JMR</i>
Source		Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 7, 1994
Composition/Purity		Test End Date	July 11, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/l

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	7.2	7	7.1	7	
	Control		7.6	7.7	7.2	7.1
	Highest Concentration		7	7.3	7.2	7.1

No. Dead or Malformed						
X 100 = %						
Total Number						
		Mortality Record			Malformation Record	
FETAX Control	3 : 100	X 100 =	3%	9 : 97	X 100 =	9.3%
Solvent Control	:	X 100 =		:	X 100 =	
Control Length (mm)	9.49	Solvent Control Length (mm)				
Minimum Concentration to Inhibit Growth (MCIG)	0.6	mg/l				

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used			
NOEL	0.05	0.1	T-test			
LOEL	1	0.2	T-test			
LC50	2.239	EC50		0.312		
95% CL	1.723	--	2.911	95% Confidence limits	0.276	---- 0.353
Test Teratogenic Index (TI = LC50/EC50): 7.18						
95% Confidence limits				5.37	--	9.59

Percent effect	LC	EC
5	0.8367732	0.162
16	1.234964	0.21
50	2.2394243	0.312
84	4.0608643	0.464
95	5.9932858	0.602

FETAX Summary Sheet

Test No. 1

Test Material	P3F	Investigator	Mendi A. Hull <i>MB</i>
Source	B5	Laboratory	OSU/Bantle
CAS No.	B4	Lot No.	E5
Test Start Date:	11/8/93	Test End Date	11/12/93
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/L
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.5	7.5	7.5	7.5	
Control		7.6	7.5	7.4	7.6
Highest Concentration		7.7	8	8	7.9

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	10 : 100 X 100 = 10%	6 : 90 X 100 = 6.7%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	0.9	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	0.2	mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.4	0.1	T-test
LOEL	0.6	0.2	T-test
LC50	0.634	EC50	0.147
95% CL	0.479 -- 0.838	95% Confidence limits	0.115 ---- 0.187
Test Teratogenic Index (TI = LC50/EC50):		4.32	
95% Confidence limits		2.98 -- 6.26	

FETAX Summary Sheet

Test No. 2

Test Material	P3F	Investigator	Mendi A. Hull <i>MAB</i>
Source	B5	Laboratory	OSU/Bantle
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	11/19/93
Solvent	B7	Conc.	E7
		Test End Date	11/23/93
		Test Units (i.e., mg/ml)	mg/L

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	7.5	7.5	7.5	7.5	
	Control		7.5	7.4	7.4	7.8
	Highest Concentration		7.8	7.6	7.8	7.7

No. Dead or Malformed			
X 100 = %			
Total Number	Mortality Record	Malformation Record	
	9 : 100 X 100 = 9%	5 : 91 X 100 = 5.5%	
	: X 100 =	: X 100 =	
FETAX Control			
Solvent Control			
Control Length (mm)	0.9	Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)		0.4	mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.4	0.1	T-test
LOEL	0.6	0.2	T-test
LC50	0.788	EC50	0.243
95% CL	0.605 -- 1.027	95% Confidence limits	0.180 ---- 0.326
Test Teratogenic Index (TI = LC50/EC50):			3.25
95% Confidence limits			2.18 -- 4.83

FETAX Summary Sheet

Test No. 3

Test Material	P3F	Investigator	Mendi A. Hull <i>MPB</i>
Source	B5	Laboratory	OSU/Bantle
CAS No.	B4	Lot No.	E5
Test Start Date:	12/2/93	Test End Date	12/6/93
Composition/Purity	C6	Test Units (i.e., mg/ml)	mg/L
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.5	7.5	7.5	7.5	
Control		7.6	7.5	7.5	7.6
Highest Concentration		7.5	7.4	7.4	7.5

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

1 : 100 X 100 = 1% 7 : 99 X 100 = 7.1%

: X 100 = : X 100 =

Control Length (mm) 0.9

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) ~~0.2~~ 0.2 mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.6	0.01	T-test
LOEL	4	0.1	T-test
LC50	0.887	EC50	0.218
95% CL	0.681 -- 1.154	95% Confidence limits	0.156 ---- 0.303

Test Teratogenic Index (TI = LC50/EC50): 4.08

95% Confidence limits 2.67 -- 6.22

FETAX SUMMARY SHEET

Test Material: P3F		Test No. Definitive #/
Source: Bantle		Investigator: Gillett/Wilborn
CAS No. — Lot No. —	Laboratory: CERL	
Composition / Purity: —		Test Start Date: 01/06/94
Solvent: — Conc. —		Test End Date: 01/10/94
		Test Units: (i.e., mg/ml) mg/l

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.4	7.0	7.6	7.5	7.4 X
Control	X	7.5	6.9 ⁹ 7.1	7.4	7.4
Highest Conc.	X	7.6	6.9	7.4	7.2

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	12 : 100 X 100 = 12%	6 : 88 X 100 = 6.8%
Solvent Control: N.A.	— : — X 100 = —%	— : — X 100 = —%
Control Length: 9.32 mm	Solvent Control Length: N.A.	mm
Minimum Concentration to Inhibit Growth (MCIG): .005		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.1 mg/L	0.01 mg/L	Williams
LOEL	0.5 mg/L	0.025 mg/L	Williams
LC ₅₀ 0.29 mg/L (Spremon-Karber)		EC ₅₀ 0.08 mg/L (Spearmon-Karber)	
95% Confidence Limits 0.22 - 0.38 mg/L		95% Confidence Limits 0.05 - 0.12 mg/L	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) - 3.625

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	— : — X 100 = —%	— : — X 100 = —%
2.5 mg/ml	— : — X 100 = —%	— : — X 100 = —%

FETAX SUMIVIARY SHEET

Test Material: P3F	Test No. Definitive #2
Source: Bottle	Investigator: Gill et
CAS No. — Lot No. —	Laboratory: CERL
Composition / Purity: —	Test Start Date: 01/14/94
Solvent: — Conc. —	Test End Date: 01/18/94
	Test Units: (i.e., mg/ml) mg/l

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.0	7.2	7.5	7.1	X
Control	+	7.5	7.3	7.4	7.5
Highest Conc.	X	7.6	7.2	6.6	7.0

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malformed X 100 = % Total Number	13:100 X 100 = 13%	16:87 X 100 = 18%
Solvent Control: N.A.	—:— X 100 = —%	—:— X 100 = —%
Control Length: 88.28 mm	Solvent Control Length: N.A. mm	
Minimum Concentration to Inhibit Growth (MCIG): 0.025		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.1 mg/L	0.05 mg/L	Williams
LOEL	0.5 mg/L	0.075 mg/L	Williams
LC ₅₀ 0.50 mg/L (Spearman-Kärber)	EC ₅₀ 0.11 mg/L (Spearman-Kärber)		
95% Confidence Limits 0.38-0.65 mg/L		95% Confidence Limits 0.06-0.19 mg/L	

$$\text{TEST TERATOGENIC INDEX (TI) = LC}_{50}/\text{EC}_{50} = 4.545$$

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	—:— X 100 = —%	—:— X 100 = —%
2.5 mg/ml	—:— X 100 = —%	—:— X 100 = —%

FETAX SUMMARY SHEET

Test Material: <u>P3F</u>		Test No. <u>Definitive #3</u>
Source: <u>Bottle</u>		Investigator: <u>Gillett</u>
CAS No. <u> </u> Lot No. <u> </u>	Laboratory: <u>CERL</u>	
Composition / Purity: <u> </u>		Test Start Date: <u>01/28/94</u>
Solvent: <u> </u> Conc. <u> </u>		Test End Date: <u>02/01/94</u>
		Test Units: (i.e., mg/ml) <u>mg/L</u>

--pH--	Day 1	Day 2	Day 3	Day 4	Day 5
Stock	7.2	7.3	7.2	7.4	X
Control	X	7.4	7.3	7.3	7.4
Highest Conc.	X	6.8	6.7	6.9	7.3

FETAX CONTROL	MORTALITY	MALFORMATION
<u>No. Dead or Malformed</u> X 100 = % Total Number	<u>2</u> : <u>100</u> X 100 = <u>2</u> %	<u>1</u> : <u>98</u> X 100 = <u>1</u> %
Solvent Control: <u>N.A.</u>	<u>—</u> : <u>—</u> X 100 = <u>—</u> %	<u>—</u> : <u>—</u> X 100 = <u>—</u> %
Control Length: <u>9.475</u> mm	Solvent Control Length: <u>N.A.</u> mm	
Minimum Concentration to Inhibit Growth (MCIG): <u>0.075</u> mg/L		

STATISTICAL RESULTS:

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.1 mg/L</u>	<u>0.075 mg/L</u>	<u>Williams</u>
LOEL	<u>0.5 mg/L</u>	<u>0.1 mg/L</u>	<u>Williams</u>
LC ₅₀ <u>0.58 mg/L</u> (Spearman-Kärber)		EC ₅₀ <u>0.41 mg/L</u> (Spearman-Kärber)	
95% Confidence Limits <u>0.51-0.66 mg/L</u>		95% Confidence Limits <u>0.19-0.86 mg/L</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀/EC₅₀) = 1.415

POSITIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
0.0055 mg/ml	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2.5 mg/ml	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

FETAX SUMMARY SHEET

Test No. 1

Test Material	UNKNOWN P3F	Investigator	TURLEY
Source	OSU	Lab	UMD - WREC
CAS No.	Lot No.	Test Start Date	11/26/93
Composition/Purity		Test End Date	11/30/93
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.13	7.11	7.15	7.13	
Control	7.65	7.60	7.67	7.70	
Highest Conc.	7.29	7.33	7.30	7.31	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number	2 : 100 X 100 = 2 %	6 : 98 X 100 = 6.1 %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length 1002 mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) 0.75 mg/L		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.50	0.75	Bonferroni T-test
LOEL	0.75	1.00	Bonferroni T-test
LC ₅₀ 1.70	Trimmed Spearman-Kärber	EC ₅₀ 2.47	Trimmed Spearman-Kärber
95% Confidence limits 1.34 - 2.15		95% Confidence limits 0.32 - 19.30	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 0.69

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX SUMMARY SHEET

Test No. 2

Test Material	UNKNOWN P3F	Investigator	TURLEY
Source	1511	Lab	UMD-WREC
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/20/93
Solvent		Test End Date	12/24/93
		Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.16	7.12	7.14	7.20	
Control	7.71	7.69	7.69	7.66	
Highest Conc.	7.20	7.23	7.26	7.23	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	1 : 80 X 100 = 1.25%	4 : 79 X 100 = 5.1%
Solvent Control	: X 100 = %	: X 100 = %
Control Length 9.29 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) 1.0		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	1.0	Bonferroni T-test
LOEL	FAIL HOMOGENEITY	3.0	Bonferroni T-test
LC ₅₀ 2.07	Trimmed Spearman-Kärber		EC ₅₀ 1.61 Trimmed Spearman-Kärber
95% Confidence Limits 1.77-2.41			95% Confidence Limits 1.21-2.14
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			1.33 / 1.29

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>UNKNOWN P3F</u>	Investigator <u>TURCEY</u>
Source <u>OSU</u>	Lab <u>UMD-WRE</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>12/23/77</u>
Solvent	Conc.
	Test End Date <u>12/27/77</u>
	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	7.09	7.17	7.13	7.16	
Control	7.77	7.69	7.69	7.73	
Highest Conc.	7.30	7.28	7.30	7.31	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>1</u> : <u>100</u> X 100 = <u>1</u> %	<u>6</u> : <u>99</u> X 100 = <u>6.1</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length <u>9.69</u> mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>1.0</u> mg/L		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	1.0	2.0	Bonferroni T-test
LOEL	2.0	3.0	Bonferroni T-test
LC ₅₀ <u>2.74</u>	Trimmed Spearman-Kärber		EC ₅₀ <u>3.95</u>
95% Confidence limits <u>1.97-3.65</u>		95% Confidence Limits <u>3.13-4.99</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 0.69

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX Summary Sheet

Test No. 1

Test Material	P3F	Investigator	Doug Dawson
Source		Laboratory	Ashland Univ
CAS No.	Lot No.	Test Start Date:	7/12/94
Composition/Purity		Test End Date	7/16/94
Solvent	Stock Co 2.5 mg/	Test Units (i.e., mg/ml)	mg/L

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.94	9.63	6.93	6.9	
Control		7.33	7.24	7.4	7.27
Highest Concentration		7.48	7.42	7.4	--

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 100 X 100 = 0%	2 : 100 X 100 = 2.0%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm) C20	Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG) G21	0.25	mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.625	T-test
LOEL	0.375	0.75	T-test
LC50	0.883	EC50	0.808
95% CL	0.791 -- 0.986	95% Confidence limits	0.722 ---- 0.905
Test Teratogenic Index (TI = LC50/EC50):			1.09
95% Confidence limits			0.93 -- 1.28

Percent effect	LC	EC
5	0.4607641	0.448
16	0.5958838	0.565
50	0.8829404	0.808
84	1.3082815	1.156
95	1.6919368	1.46

FETAX Summary Sheet

Test No. 2

Test Material	P3F	Investigator	Doug Dawson
Source		Laboratory	Ashland Univ
CAS No.	Lot No.	Test Start Date:	7/18/94
Composition/Purity		Test End Date	7/22/94
Solvent	Stock Co 2.5 mg/	Test Units (i.e., mg/ml)	mg/L

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.07	7.07	7.07	7.1	
Control		7.44	7.41	7.3	7.19
Highest Concentration		7.5	7.43	7.4	7.27

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm)

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

0 : 100

X 100 =

0%

3 : 100

X 100 =

3.0%

:

X 100 =

:

X 100 =

Solvent Control Length (mm)

0.25

mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.375	T-test
LOEL	0.625	0.75	T-test
LC50	0.815	EC50	0.759
95% CL	0.736	--	0.902
95% Confidence limits		0.675	0.855

Test Teratogenic Index (TI = LC50/EC50):

1.07

95% Confidence limits

0.92

--

1.25

Percent effect	LC	EC
5	0.4879727	0.466
16	0.5976449	0.565
50	0.8148417	0.759
84	1.1109725	1.02
95	1.3606643	1.238

FETAX Summary Sheet

Test No. 3

Test Material	P3F	Investigator	Doug Dawson
Source		Laboratory	Ashland Univ
CAS No.	Lot No.	Test Start Date:	7/21/94
Composition/Purity		Test End Date	7/25/94
Solvent	Stock Co 2.5 mg/	Test Units (i.e., mg/ml)	mg/L

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.09	7.07	7.11	7.1	
Control		7.51	7.42	7.3	7.16
Highest Concentration		7.42	7.39	7.3	7.29

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm)

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

0 : 100

X 100 =

0%

4 : 100

X 100 =

4.0%

:

X 100 =

:

X 100 =

Solvent Control Length (mm)

0.1

mg/L

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.375	T-test
LOEL	0.625	0.5	T-test
LC50	0.933	EC50	0.802
95% CL	0.831	--	1.049
95% Confidence limits		0.709	0.908

Test Teratogenic Index (TI = LC50/EC50): 1.16

95% Confidence limits 0.98 -- 1.38

Percent effect	LC	EC
5	0.5032428	0.418
16	0.6425083	0.541
50	0.9334985	0.802
84	1.3562773	1.189
95	1.7316082	1.538

FETAX SUMMARY SHEET

Test No. 1

Test Material	CuSO ₄ P3F	Investigator	M. JACKSON
Source		Lab	STOVER BIO LAB
CAS No.		Test Start Date	8/24/93
Lot No.		Test End Date	8/28/93
Composition/Purity		Test Units (i.e., mg/ml)	mg/L
Solvent		Conc.	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.8	7.8	7.8	7.8	
Control	7.8	7.8	7.8	7.8	
Highest Conc.	7.8	7.8	7.8	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	7 : 100 X 100 = 7 %	4 : 93 X 100 = 4 %
Solvent Control NA	: X 100 = %	: X 100 = %
Control Length 90.9 mm	Solvent Control Length NA mm	
Minimum Concentration to Inhibit Growth (MCIG) 0.5		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	NA	Failed variance assumption
LOEL	NA	NA	"
LC ₅₀	1.49	EC ₅₀	1.48
95% Confidence limits	1.31 - 1.70	95% Confidence Limits	1.18 - 1.85
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			1.00

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

FETAX SUMMARY SHEET

Test No. 4 *

Test Material	P3P	Investigator	
Source	OSU / BANTLE	Lab	
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/10/93
Solvent		Test End Date	12/14/93
Conc.		Test Units (i.e., mg/ml)	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.4	7.4	7.4	7.4	
Control	8.0	8.0	7.9	8.1	
Highest Conc.	7.6	7.4	7.5	7.5	

FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed	X 100 = %				
Total Number		0 : 100 X 100 = 0 %		4 : 100 X 100 = 4 %	
Solvent Control		: X 100 = %		: X 100 = %	
Control Length	93.4 mm	Solvent Control Length		mm	
Minimum Concentration to Inhibit Growth (MCIG) 0.3					

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	2.09	EC ₅₀	0.89
95% Confidence limits	2.00 - 2.18	95% Confidence Limits	0.85 - 0.94

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) 2.35

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

* C_m measured = 3.9 mg/L corrected for weighing
 Gravimetric = 10.0 mg/L error

FETAX SUMMARY SHEET

Test No. 15

Test Material <u>P3F</u>	Investigator
Source <u>OSU / BANTLE</u>	Lab
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>12/10/93</u>
Solvent	Conc.
	Test End Date <u>12/14/93</u>
	Test Units (i.e., mg/ml)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.4	7.4	7.4	7.4	
Control	8.0	8.0	8.0	8.0	
Highest Conc.	7.6	7.6	7.4	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %	<u>0</u> : <u>100</u> X 100 = <u>0</u> %
Solvent Control	: X 100 = %	: X 100 = %
Control Length <u>94.4</u> mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.5</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>2.20</u>	EC ₅₀	<u>0.92</u>
95% Confidence limits	<u>2.10 - 2.51</u>	95% Confidence Limits	<u>0.84 - 1.03</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

2.39

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

* Cu measured = 3.9 mg/L
Cuv. metric = 10.0 mg/L

corrected
for error
(DE)